

Nos. 23-2074, 23-2075, 23-2191, 23-2192, 23-2193,
23-2194, 23-2239, 23-2252, 23-2253, 23-2255

**In the United States Court of Appeals
for the Federal Circuit**

CYTIVA BIOPROCESS R&D AB,
APPELLANT

v.

JSR CORP., JSR LIFE SCIENCES, LLC.,
CROSS-APPELLANTS

Appeals from the United States Patent and Trademark Office,
Patent Trial and Appeal Board in Nos. IPR2022-00036, IPR2022-00041,
IPR2022-00042, IPR2022-00043, IPR2022-00044, IPR2022-00045.

**OPENING BRIEF OF APPELLANT
CYTIVA BIOPROCESS R&D AB**

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EXEMPLARY PATENT CLAIMS

U.S. Patent No. 10,213,765

1. A chromatography matrix comprising:
a solid support; and
a ligand coupled to the solid support, the ligand comprising at least two polypeptides,
wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and
wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.
4. The chromatography matrix of claim 1, wherein the ligand is capable of binding to the Fab part of an antibody.

U.S. Patent No. 10,343,142

1. A process for isolating one or more target compound(s), the process comprising:
 - (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1; and
 - (b) adsorbing the target compound(s) to the ligand; and
 - (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand.

4. The process of claim 1, wherein the ligand binds to the Fab part of an antibody.

U.S. Patent No. 10,875,007

1. A process for isolating one or more target compound(s), the process comprising:
 - (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 52 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1; and
 - (b) adsorbing the target compound(s) to the ligand; and
 - (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand; and
 - (d) performing a cleaning in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.
11. The process of claim 1, wherein the ligand binds to the Fab part of an antibody.

CERTIFICATE OF INTEREST

Pursuant to Federal Circuit Rule 47.4, undersigned counsel for appellant certifies the following:

1. The full name of every party represented by me is Cytiva Bioprocess R&D AB.
2. Cytiva Sweden AB is the exclusive licensee of the Patents-in-Suit and an additional real party in interest represented by me.
3. Cytiva Bioprocess R&D AB and Cytiva Sweden AB are wholly-owned subsidiaries of Danaher Corporation.
4. The following attorneys appeared for Cytiva Bioprocess R&D AB in proceedings below or are expected to appear in this Court and are not already listed on the docket for the current case:
 - (a) Of Williams & Connolly LLP, 680 Maine Ave., S.W., Washington, D.C. 20024: David I. Berl, Teagan James Gregory, and Andrew L. Hoffman.
 - (b) Formerly of Williams & Connolly LLP: Sarahi Uribe.
5. The title and number of any case known to counsel to be pending in this or any other court or agency that will directly affect or be directly

affected by this Court's decision in the pending appeals is *Cytiva Bioprocess R&D AB, et al. v. JSR Corporation, et al.*, 1:21-cv-310-RGA (D. Del.).

OCTOBER 27, 2023

/s/ David M. Krinsky

DAVID M. KRINSKY

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STATEMENT OF RELATED CASES

Pursuant to Federal Circuit Rule 47.5, Cytiva Bioprocess R&D AB (“Cytiva”) states that no other appeal in or from the same *inter partes* review (“IPR”) proceedings at the Patent Trial and Appeal Board (the “Board”) was previously before this Court or any other appellate court. The Board proceedings from which these appeals arise concern U.S. Patent Nos. 10,213,765; 10,323,142; and 10,875,007. Cytiva has asserted each of these patents in a pending district court case that will be affected by this Court’s decision in the pending appeals: *Cytiva Bioprocess R&D AB, et al. v. JSR Corporation, et al.*, 1:21-cv-310-RGA (D. Del.).

JURISDICTIONAL STATEMENT

JSR Corporation and JSR Life Sciences, LLC (collectively, “JSR”) jointly filed six IPR petitions, challenging the validity of U.S. Patent Nos. 10,213,765; 10,323,142; and 10,875,007. Appx406-863. The Board had jurisdiction over the IPR proceedings pursuant to 35 U.S.C. § 6. On April 19, 2023, the Board issued Final Written Decisions in IPR2022-00036 and IPR2022-00043. Appx1-54; Appx177-230. On May 18, 2023, the Board issued Final Written Decisions in IPR2022-00041, IPR2022-00042, IPR2022-00044, and IPR2022-00045. Appx55-176; Appx231-352. Cytiva timely filed its notices of appeal on June 20, 2023, and July 19, 2023. 37 C.F.R. § 90.3(a)(1); Appx2294-2681. JSR timely filed its notices of cross-appeal on July 28, 2023. 37 C.F.R. § 90.3(a)(1); Appx2682-2945. This Court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(4)(A).

STATEMENT OF THE ISSUES

1. Whether the Patent Trial and Appeal Board erred in ruling that claims 1-7, 10-20, and 23-26 of U.S. Patent No. 10,213,765; claims 1-3, 5-7, 10-16, 18-20, and 23-30 of U.S. Patent No. 10,323,142; and claims 1-10, 12-14, 16-28, 30-32, and 34-37 of U.S. patent No. 10,875,007 are unpatentable, without performing a lead compound analysis.

2. Whether the Patent Trial and Appeal Board erred in ruling that claims 4 and 17 of U.S. Patent No. 10,213,765 are unpatentable based on a holding that an unexpected property inherently would have been obvious.

STATEMENT OF THE CASE

JSR filed six related petitions for *inter partes* review challenging most claims of U.S. Patent Nos. 10,213,765 (“the ’765 patent”), 10,323,142 (“the ’142 patent”), and 10,875,007 (“the ’007 patent”), which relate to novel compounds—protein A ligands—for use in chromatography matrices. Appx406-863. The Board granted institution on all six petitions, Appx1073-1158; Appx1171-1349, and, after an oral hearing, ruled that claims 1-7, 10-20, and 23-26 of the ’765 patent; claims 1-3, 5-7, 10-16, 18-20, and 23-30 of the ’142 patent; and claims 1-10, 12-14, 16-28, 30-32, and 34-37 of the ’007 patent are unpatentable, Appx1-352. The Board determined that JSR failed to prove by

a preponderance of the evidence that claims 4 and 17 of the '142 patent and claims 11 and 29 of the '007 patent are unpatentable. Appx55-176; Appx231-352. These appeals followed and have since been consolidated.

STATEMENT OF FACTS

A. Background

Cytiva is a global leader in the development, manufacturing, and marketing of biopharmaceutical technologies. Its technologies aid researchers developing innovative drugs and therapies as well as companies involved in large-scale manufacturing of biopharmaceuticals. One important line of products developed and marketed by Cytiva is chromatography resins, including novel ligands immobilized on resins.

These appeals concern the validity of patent claims directed to new and improved chromatography ligands developed by Cytiva and methods of using those ligands. As relevant here, chromatography ligands are chemical compounds that bind selectively to biomolecules known as antibodies. The ligands are therefore useful in isolating and purifying antibodies and antibody fragments, which are used in pharmaceutical products, among other things.

More specifically, the claims relate to chromatography matrices comprising ligands composed of the amino acid sequence of Domain C of protein A—a naturally occurring protein derived from bacteria—but modified by substituting the glycine residue at position 29 with an alanine. This sequence has been referred to by the Board and parties as a “C(G29A)” ligand, reflecting the “G29A” substitution in Domain C. Independent claim 1 of the ’765 patent is illustrative of the claimed subject matter:

1. A chromatography matrix comprising:
 - a solid support; and
 - a ligand coupled to the solid support, the ligand comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.

Appx378. SEQ ID NO. 1 sets forth the amino acid sequence of Domain C.

Appx378.¹

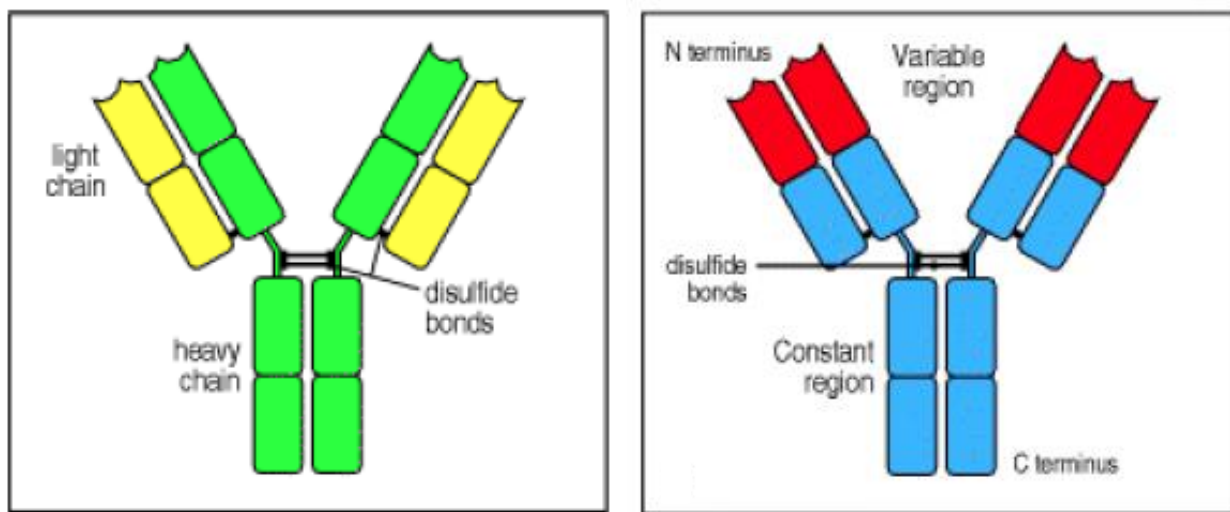
¹ Because SEQ ID NO. 1 is more than 55 amino acids long, the “at least 55 contiguous amino acids” limitation means that the “ligand” limitation encompasses a narrow genus of ligands having different amino acids at one or both ends of each polypeptide. Because these variations and the presence of multiple polypeptides per ligand are not relevant to the issues in these appeals, Cytiva will refer to all of the recited ligands collectively as “C(G29A)” ligands.

1. Antibodies and Antibody Fragments

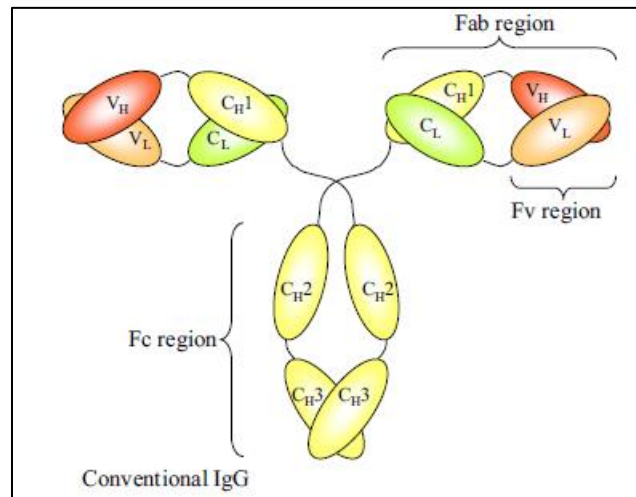
Antibodies are relatively large molecules that play an important role in the immune system's defenses against invading pathogens. Appx5801.

Antibodies are used widely in clinical and diagnostic applications. Indeed, many of today's most important therapeutics are antibodies. *See* Appx371; Appx2964; Appx7258.

Human antibodies, or immunoglobulins, come in five classes, of which immunoglobulin G (IgG) is the most prevalent. Appx5816. IgG antibodies are composed of amino acids covalently bonded to each other to form polypeptides. Appx7255. They are roughly Y-shaped and consist of two identical heavy chains and two identical light chains. Appx5839-43. These chains can be subdivided into "constant" and "variable" regions, as depicted below, Appx5840-41:



Antibodies also can be subdivided into “fragment antigen-binding” (Fab) regions and a “fragment crystallizable” (Fc) region. Appx5802-03. A Fab region contains the variable portions, or domains, of both the heavy and light chains as well as the sole constant domain of the light chain and the first constant domain of the heavy chain. Appx5802-03. The Fc region contains the second and third constant domains of both heavy chains. Appx5802-03. These regions (again in an IgG antibody) are depicted below, Appx5802:



Fab regions separated from antibodies are known as “Fab fragments,” and have their own advantageous clinical and diagnostic uses. *See* Appx5802; Appx5874-79; Appx6254 (JSR’s expert, Dr. Cramer, agreeing the person of ordinary skill in the art would “have been motivated to make ligands that b[ind] to Fab”). For instance, in addition to being easier and cheaper to manufacture, the relatively small size of Fab fragments allow them to

penetrate tissues that full antibodies cannot access and to clear from circulation more rapidly. Appx5879.

The manufacture of antibodies and their fragments is a complex process. Antibodies are grown in living cells. They must be separated from the cell culture and purified of unwanted materials. Protein A affinity chromatography is often the first step in industrial-scale antibody purification.

2. Protein A Chromatography

Protein A, also known as SPA, is a naturally occurring protein found in the bacterium *Staphylococcus aureus*. Appx3815-16. It has a high binding affinity for the Fc region of IgG. Appx3815; Appx3896. In its natural form, protein A has five domains: Domains A, B, C, D, and E. Appx3815. These domains have been studied to differing degrees. Domain B, and engineered forms of Domain B constructed by modifying the protein's amino acid sequence, have been the primary focus of protein A chromatography research. *See infra* p. 27 n. 4; Appx7261, Appx7264-65.

Protein A chromatography takes advantage of protein A's strong affinity for antibodies. Protein A (or a particular protein A domain) is immobilized to a resin that is packed into a chromatography column. The

composition containing the target antibody is then loaded onto (*i.e.*, pumped or injected into) the column. Appx2970; Appx7261-62. As the composition passes through the column, the target antibody selectively binds to protein A, thus separating the antibody from the rest of the mixture. Appx2970; Appx7261-62. Column conditions are then changed—typically by introducing a low-pH solution—to dissociate the target antibody from protein A, leading to elution of the now-purified antibody. Appx2970; Appx7261-62.

Protein A-based chromatography columns are expensive to manufacture, so the columns are designed to permit reuse over multiple cycles of antibody purification. Appx2971-72; Appx7262-63. To facilitate repeat use, protein A columns are cleaned between operations, typically using a highly alkaline solution in a step referred to as cleaning-in-place. Appx2971-72; Appx7262-63. Those same alkaline solutions, however, can degrade the protein A ligands and impair their ability to bind antibodies or antibody fragments. Appx2971-72; Appx7262-63. While natural protein A is fairly stable, industry and research groups have worked to develop protein A ligands capable of better retaining binding capacity after the harsh cleaning-in-place step.

3. Protein A Engineering

One method of improving protein A's resistance to alkaline degradation is protein engineering, or the process of changing protein A's amino acid sequence. Appx7263. As of the priority date in 2006 (and even today), however, protein engineering was a complex and unpredictable art. Appx7263. It is nearly impossible to predict the effects of even single amino acid mutations. *See* Appx6260 (Dr. Cramer stating that determining the impacts of amino acid substitutions on antibody binding “would require a detailed analysis”); Appx6261 (Dr. Cramer opining that “it would be very difficult to predict . . . a priori” what effect a mutation would have on antibody binding); Appx6266 (Dr. Cramer stating “[i]t would be difficult to predict” whether a single amino acid change to one SPA domain would have the same effect on antibody binding if that change were made to a different SPA domain). As the patent examiner explained during prosecution of the '765 patent, “[p]rotein chemistry is probably one of the most unpredictable areas of biotechnology,” and “the effects of sequence dissimilarities upon protein structure and function cannot be predicted.” Appx7361.

This complexity and unpredictability is aptly demonstrated by the teachings of Jansson. *See* Appx7368-77. Jansson examines the Fab binding

of the five naturally occurring SPA domains as well as “Domain Z,” an engineered form of Domain B with a glycine substituted for alanine at amino acid position 29 (commonly denoted “G29A”). It was known that a G29A mutation to Domain B improved the domain’s alkaline stability. *See, e.g.,* Appx3911. Jansson found, however, that “domain B clearly binds to Fab whereas the very similar domain Z shows only little or no binding activity.” Appx7375. Put differently, the very G29A mutation to Domain B that improved alkaline stability also impaired Fab binding.

Before the priority date of 2006, little or no protein engineering had been done with protein A’s Domain C. Appx7265; *see also* Appx6309-10 (Dr. Cramer unable to recall or cite any experimental data involving mutated versions of Domain C). Instead, nearly all research efforts associated with optimizing protein A-based ligands for use in affinity chromatography concerned Domain B and the engineered Domain B analog, Domain Z. *See infra* p. 27 n. 4. The extensive use of Domain B can be credited at least in part to publication of the crystal structure of Domain B in complex with the Fc region of an IgG antibody, and the fact that Domain B “is the closest to a hypothetical consensus sequence of the IgG-binding domains.” Appx4154; Appx7265 (citing Appx4152-61). Domain B- and Z-based resins also were

sold as commercial products, including the alkaline-stable MabSelect SuRe™ product sold by Cytiva’s predecessor, GE Healthcare. Appx375.

B. Proceedings Below

1. In early 2021, Cytiva sued JSR for infringement of the ’765, ’142, and ’007 patents in the United States District Court for the District of Delaware. *Cytiva Bioprocess R&D AB et al. v. JSR Corp. et al.*, 1:23-cv-00310-RGA, D.I. 1 (D. Del. Feb. 26, 2021). Cytiva accused JSR of marketing an infringing protein A chromatography resin under the trade name Amsphere™ A3. *See id.* Because Cytiva has developed and markets competing protein A chromatography resins, Cytiva sought permanent injunctive relief in addition to damages. *See id.*

2. About eight months later, JSR filed six *inter partes* review (“IPR”) petitions challenging claims 1-7, 10-20 and 23-26 of the ’765 patent (IPR2022-00036 and IPR2022-00043); claims 1-7, 10-20, and 23-30 of the ’142 patent (IPR2022-00041 and IPR2022-00044); and claims 1-14, 16-32, and 34-37 of the ’007 patent (IPR2022-00042 and IPR2022-00045). Appx406-863. The Patent Trial and Appeal Board (the “Board”) granted institution on all six petitions, Appx1073-1158; Appx1171-1349, and the district court action

was stayed, *Cytiva Bioprocess R&D AB et al. v. JSR Corp. et al.*, 1:23-cv-00310-RGA, D.I. 67 (D. Del. May 11, 2022).

The grounds for the institution decisions largely overlapped. As relevant here, the petitions argued that all challenged claims were rendered obvious under 35 U.S.C. § 103 by Martin Linhult et al., “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach,” *PROTEINS: STRUCTURE, FUNCTION, AND BIOINFORMATICS* vol. 55 pp. 407-416 (2004) (“Linhult”), Appx3815-24; U.S. Patent No. 5,143,844 (“Abrahmsén”), Appx3825-38; and International Publication No. WO 03/080655 (“Hober”), Appx3839-95. In support of the petitions, JSR filed declarations from its expert, Dr. Steven M. Cramer. Appx2946-3781. Cytiva filed a competing declaration from its expert, Dr. Daniel Bracewell. Appx7111-7240.

Linhult and Hober each disclose the results of protein engineering work intended to improve the alkaline stability of a Domain Z-based SPA ligand to allow it to withstand longer exposure to the harsh alkaline conditions imposed by the cleaning-in-place step. Appx3815-24; Appx3839-95. Specifically, both Linhult and Hober report the results of stability studies performed on various mutated forms of Domain Z replacing

asparagine residues. Neither Linhult nor Hober describes efforts to modify Domain C or to replace glycine residues in SPA-based ligands. Likewise, neither reference describes the Fab-binding characteristics of the engineered Domain Z-based ligands.

Abrahmsén is a 1992 patent that describes a purification process for antibodies using an affinity chromatography column with immobilized IgG ligands. Appx3825-38. That process consists of fusing protein A to the target antibody and then running the fusion protein through the IgG column to remove impurities, cleaving to separate the protein A from the target antibody, and re-running through the IgG column to remove the now-separated protein A from the composition containing the target antibody. Appx3825-38. The SPA domain of interest throughout Abrahmsén is Domain Z, and the claimed invention is a recombinant DNA fragment coding for Domain Z and a second heterologous protein. Appx3825-38. Abrahmsén does not discuss protein engineering strategies for SPA-based ligands, report efforts to modify Domain C, or describe the Fab-binding properties of engineered ligands.

The six petitions' overlapping obviousness arguments all followed the same general formula. They each observed the prior art's disclosure of

Domain C's amino acid sequence, of the sensitivity of asparagine-glycine dipeptide sequences to alkaline conditions, and of G29A mutations outside of Domain C—either in the context of Domain B or generally with respect to any one of the naturally occurring SPA domains. The petitions then argued that the person of ordinary skill in the art (“POSA”) would have found it obvious to combine these disclosures to reach the claimed ligands.² None of the petitions argued that the POSA would have been motivated to select Domain C over other domains as the foundation for an engineered SPA ligand. Instead, JSR argued in its papers that a G29A modification to any one of the five, naturally occurring SPA domains was among a finite number of predictable solutions—despite the unpredictability of protein engineering

² Three of the six petitions—one per patent—relied on a fourth reference, U.S. Patent Publication 2006/0134805 (“Berg”), Appx4162-71, and argued that passing references to Domain C suggested the POSA would have started with the amino acid sequence of that domain when designing an SPA-based ligand with improved alkaline stability. Cytiva responded that Berg focused not on Domain C, but rather on Domains B and Z, and in any event was a patent application relating to improved resin particles rather than any development in the field of protein A ligands. Appx7290-93; Appx6295-96 (Dr. Cramer agreeing that Berg is directed to engineering the chromatography resin, or solid support, rather than protein A ligands). The parties also disputed whether Berg qualified as prior art under 35 U.S.C. § 103, and the Board explicitly did not reach the grounds that relied on Berg. *E.g.*, Appx109-11; Appx171-72; Appx227.

and the prior art's uniform focus on Domain B or its modified form, Domain Z. *See, e.g.*, Appx436-40; Appx650-53; Appx811-14; Appx1787-91.

JSR's petitions also took aim at dependent claims relating to, *inter alia*, the Fab-binding properties of the claimed C(G29A)-based matrices. Relevant here, in IPR2022-00036 and IPR2022-00043, JSR challenged the validity of dependent claims 4 and 17 of the '765 patent, which recite the matrices of claims 1 and 14, respectively, "wherein the ligand is capable of binding to the Fab part of an antibody." Appx378-79. Cytiva argued that none of JSR's prior art references described a C(G29A)-based SPA ligand, let alone suggested that such a ligand would have the claimed Fab-binding properties. *See, e.g.*, Appx1428-32; Appx2065-72. In fact, if the POSA were to glean anything about the properties of a C(G29A)-based ligand from the prior art, the POSA would have expected such a ligand to *lack* Fab-binding ability—the exact opposite of what the claims require. *See, e.g.*, Appx1428-32; Appx2065-72. Pointing to Jansson, Cytiva observed that the G29A mutation (at least in Domain B) destroys Fab binding. *See, e.g.*, Appx1428-32; Appx2065-72. Cytiva also noted other references that reinforce this conclusion. *See, e.g.*, Appx2065-70 (discussing Appx7474-80; Appx6231-36).

The parties disagreed as to whether inherency could be used to fill a gap in a reasonable expectation of success analysis and whether the unexpected Fab binding of a C(G29A)-based ligand can support a finding of nonobviousness. JSR argued that Fab-binding is an inherent property of a C(G29A)-based ligand and that necessarily present properties do not add patentable weight to claims. *See, e.g.*, Appx1800-02, Appx1807; Appx1831-33, Appx1838; Appx1862-64, Appx1870. Cytiva argued that the mere fact that a certain thing may result from a given set of circumstances is not sufficient to render the result obvious; a patent challenger must still establish that a claim limitation (here Fab binding) was foreseen or expected. *See, e.g.*, Appx1431-32; Appx2070-72. And the evidence of record here, if anything, established just the opposite, supporting the nonobviousness of the challenged claims. *See, e.g.*, Appx1428-31, Appx1443; Appx1493-96, Appx1499-1500; Appx1559-62, Appx1565.

3. In a consolidated final written decision, the Board found all challenged claims of the '765 patent unpatentable. Appx1-54; Appx177-230. As to the '142 and '007 patents, the Board upheld the dependent Fab-binding claims (claims 4 and 17 of the '142 patent and claims 11 and 29 of the '007 patent) but found the other challenged claims unpatentable. Appx55-115;

Appx116-76; Appx231-91; Appx292-352. As to the upheld claims, the Board held that because the prior art “does not support the conclusion that [a] G29A mutation in a SPA domain ligand binds Fab, [JSR] has not established by a preponderance of the evidence of record that the process of isolating Fab target[s] using a mutated SPA domain C ligand would have been obvious.” Appx104-05; Appx166; Appx280-81; Appx341.

The Board’s decisions proceeded in similar fashion. The Board first reviewed Linhult, Abrahmsén, and Hober, fixating on references to all five SPA domains as well as disclosures that an asparagine-glycine dipeptide sequence is sensitive to degradation under alkaline conditions. *See* Appx33-34; Appx87-89; Appx149-51; Appx209-10; Appx263-66; Appx325-28. From these general teachings, the Board found that the prior art “suggest[s] mutating the glycine at position 29 for an alanine in *any one* of the SPA IgG binding domains of E D A B or C in order to avoid protein degradation in alkaline conditions and degradation by hydroxylamine.” Appx34; Appx210; Appx327-28 (emphasis added); *see also* Appx90; Appx151; Appx266 (same finding).

The Board did not dispute that, as of the priority date, no one was working with Domain C. *See, e.g.*, Appx1396-1411; Appx7284-98. Nor did the

Board dispute that the field of protein engineering is notoriously unpredictable. *See, e.g.*, Appx1396-1411; Appx7263-69. Yet rather than perform the well-established lead compound analysis typical in new chemical compound cases, the Board relied on JSR's proffers of sequence homology and passing references to making the G29A mutation in *any one* of SPA's domains to establish obviousness. *See, e.g.*, Appx35-38; Appx91-93; Appx152-54; Appx211-14; Appx267-69; Appx328-30.

As to the dependent Fab-binding claims of the '765 patent (claims 4 and 17), the Board refused to consider evidence of the surprising Fab-binding capacity of the claimed C(G29A) ligand. The Board cited no evidence establishing that the POSA would have had a reasonable expectation of success in practicing the challenged claims. Instead, the Board pointed to the alleged inherency of Fab binding to fill the gap in its obviousness analysis. *See, e.g.*, Appx44-48; Appx220-24. The Board applied the same reasoning in rejecting Cytiva's evidence of unexpected results supporting the non-obviousness of the challenged claims. *See, e.g.*, Appx48-49; Appx224-25.

4. The district court action against JSR—filed over two-and-a-half years ago—remains stayed pending resolution of these appeals. *Cytiva Bioprocess R&D AB et al. v. JSR Corp et al.*, 1:21-cv-00310-RGA, D.I. 90 (D.

Del. Aug. 11, 2023). Given the impact of the stay on Cytiva's ability to obtain relief from the harm created by JSR's competing and infringing protein A chromatography resin, Cytiva respectfully requests that the Court resolve these appeals expeditiously. To assist in that end, Cytiva is filing this opening brief in advance of the deadline.

SUMMARY OF ARGUMENT

I. The Board's determination that claims 1-7, 10-20, and 23-26 of the '765 patent, claims 1-3, 5-7, 10-16, 18-20, and 23-30 of the '142 patent, and claims 1-10, 12-14, 16-28, 30-32, and 34-37 of the '007 patent would have been obvious should be reversed because the Board failed to assess whether—and JSR failed to present evidence that—the POSA would have selected Domain C as a lead compound over Domains B and Z.

A. The ligands of the claimed inventions are novel chemical compounds obtained by making a G29A modification to the prior-art Domain C. JSR was required but failed to demonstrate the POSA would have selected Domain C as a lead compound for modification.

B. The POSA would not have selected Domain C as a lead compound over the consensus favorites Domains B and Z or the modified

forms of Domains B and Z described in the art, and JSR presented no evidence to the contrary.

C. The Board's "obvious to try" assessment cannot substitute for the required lead compound analysis.

II. The Board's determination that claims 4 and 17 of the '765 patent are obvious should be reversed because the Board failed to account properly for the unexpected Fab-binding property recited in those claims.

A. The Board failed to find or even consider whether the POSA would have a reasonable expectation of success in achieving the claimed Fab-binding with the C(G29A)-based ligands of the claimed inventions. The Board erroneously substituted its finding that Fab-binding is an inherent property of C(G29A)-based ligands for the required reasonable expectation of success analysis.

B. The Board failed to consider whether the Fab-binding of the claimed ligands reflected an unexpected result indicative of nonobviousness. The Board erroneously substituted its inherency conclusion for a proper analysis of objective indicia of nonobviousness.

STANDARD OF REVIEW

This Court “review[s] the Board’s compliance with the governing legal standards de novo.” *Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015). “Obviousness is a mixed question of fact and law” and “[a]lthough the Board’s ultimate [obviousness] conclusion . . . is a legal determination subject to de novo review, the subsidiary factual findings are reviewed for substantial evidence.” *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1366 (Fed. Cir. 2016). Typically, the “presence or absence of a reasonable expectation of success is . . . a question of fact,” *id.*, but the “failure to consider the appropriate scope of the . . . claimed invention in evaluating the reasonable expectation of success . . . constitutes a legal error that [this Court] review[s] without deference,” *Allergan, Inc. v. Apotex Inc.*, 754 F.3d 952, 966 (Fed. Cir. 2014).

ARGUMENT

I. The Board Erred in Failing to Perform a Lead Compound Analysis

The Board, at JSR’s urging, skipped a critical step in its obviousness analysis of all claims it held unpatentable. Rather than require JSR to identify a reason to select Domain C as a starting point from the sea of prior art teaching a preference for Domain B and the related Domain Z, the Board

permitted JSR to rely on general teachings that any one of the five naturally occurring SPA domains can function as a ligand for the purification of whole antibodies. Its approach ignores the foundational principle of obviousness that the POSA must have a reason to carry out the claimed invention. *See, e.g., KSR Int'l Co. v. Teleflex, Inc.*, 550 U.S. 398, 418 (2007). It also traduces decades of precedent from this Court applying this principle to the chemical arts, holding that a compound that reflects a modification of a prior-art compound would have been obvious only if the POSA had a reason to start with the prior-art compound and modify it to reach the one that is claimed. *See, e.g., Otsuka Pharm. Co. v. Sandoz, Inc.*, 678 F.3d 1280, 1291 (Fed. Cir. 2012). Even though the claimed compound here is a Domain C ligand modified with a G29A substitution, JSR did not even attempt to argue that the POSA would have started with Domain C to design an SPA-based ligand with improved alkaline stability. Nor did JSR proffer any other route by which the POSA would modify a prior-art compound and obtain the claimed invention. Instead, *all* of JSR's proffered art concerns Domains B or Z, and the Board's refusal to enforce a lead compound requirement was legal error warranting reversal.

A. A Lead Compound Analysis is Required Where the Purported Invention Is a Chemical Compound That Is a Modified Version of a Prior-Art Compound.

The Board erred in assessing the obviousness of the challenged claims by applying the wrong legal framework. At bottom, the challenged claims concern new chemical compounds—specifically, modified versions of a known compound, Domain C. *See, e.g.*, Appx378 ('765 patent claim 1); Appx391 ('142 patent claim 1); Appx404 ('007 patent claim 1). As this Court has explained: “[W]hether a new chemical compound would have been *prima facie* obvious over particular prior art compounds” begins by determining whether the POSA “would have selected the asserted prior art compounds as lead compounds, or starting points, for further development efforts.” *Otsuka*, 678 F.3d at 1291; *Procter & Gamble Co. v. Teva Pharms. USA, Inc.*, 566 F.3d 989, 994 (Fed. Cir. 2009) (“An obviousness argument based on structural similarity between claimed and prior art compounds ‘clearly depends on a preliminary finding that one of ordinary skill in the art would have selected [the prior art compound] as a lead compound.’”).

A “lead compound” is “a compound in the prior art that would be most promising to modify in order to improve upon its . . . activity and obtain a compound with better activity.” *Otsuka*, 678 F.3d at 1291 (quoting *Takeda*

Chem. Indus., Ltd. v. Alphapharm Pty., Ltd., 492 F.3d 1350, 1357 (Fed. Cir. 2007)). “In determining whether [the POSA] would have selected a prior art compound as a lead, the analysis is guided by evidence of the compound’s pertinent properties” and comparison against other prior art compounds. *Id.* at 1292. “Absent a reason or motivation based on such prior art evidence, mere structural similarity between a prior art compound and the claimed compound does not inform the lead compound selection.” *Id.* A patent challenger must show the POSA had “a reason to select a proposed lead compound or compounds *over* other compounds in the prior art.” *Daiichi Sankyo Co. v. Matrix Labs., Ltd.*, 619 F.3d 1346, 1354 (Fed. Cir. 2010) (emphasis added).

This Court explained in *Otsuka* why the lead compound analysis is important when considering the obviousness of chemical compounds that are based on modifying known compounds: it shields against “impermissibl[e] . . . *ex post* reasoning.” *Otsuka*, 678 F.3d at 1292. Many inventions in the chemical arts involve relatively small structural changes to prior-art molecules, and focusing solely on the “structural similarity between claimed and prior art subject matter,” without consideration of whether the POSA would have reason to select the particular starting point that leads to the

claimed invention, risks importing hindsight bias to make the invention seem obvious. The case law therefore has long required courts finding obviousness to determine that “the prior art gives reason or motivation to make the claimed compositions,” *In re Dillon*, 919 F.2d 688, 692 (Fed. Cir. 1990) (en banc); see *Otsuka*, 678 F.3d at 1292 (quoting *Dillon*)—typically, by establishing that the POSA would have “selected a prior art compound as a lead,” *Otsuka*, 678 F.3d at 1292. *Otsuka*’s framework is “well-established” law, and part and parcel of proving the POSA would have been motivated to modify a known compound to arrive at the claimed invention. See *Takeda*, 492 F.3d at 1356. Without such motivation, the claimed invention cannot be obvious. *Id.* at 1357.

B. The POSA Would Not Have Been Motivated to Create a Chromatography Matrix Comprising a Domain C-Based SPA Ligand.

1. Under the “well-established” lead compound framework, it was JSR’s burden below to compare Domain C against other prior art compounds and, based on comparison of the prior art compounds’ known properties or other teachings in the art, to demonstrate that the POSA would have selected Domain C for modification over other compounds, such as Domains B or Z. See *Daiichi Sankyo Co.*, 619 F.3d at 1354. JSR, however, skipped

this step. Nowhere in its petitions did JSR even attempt to explain why the POSA would have selected Domain C for modification over Domains B or Z to arrive at the claimed C(G29A) ligand. The Board committed the same legal error. And because, under the correct legal standard, JSR presented no evidence that the POSA would have selected Domain C as a “lead” over other compounds, the Board’s decision must be reversed. *See Arendi S.A.R.L. v. Apple Inc.*, 832 F.3d 1355, 1366 (Fed. Cir. 2016) (reversing the Board’s obviousness determination and noting that “this is not a case where a more reasoned explanation than that provided by the Board can be gleaned from the record”).

Rather than perform the required lead compound analysis, the Board concluded that the prior art taught replacing the glycine at position 29 with an alanine in *any one* of five SPA binding domains. *See* Appx35-38; Appx91-93; Appx152-54; Appx211-14; Appx267-69; Appx328-30. But the prior art offers no reason to prefer Domain C to the consensus favorites: Domains B and Z. The Board relied on fleeting references to all five naturally occurring SPA domains to supply the requisite motivation. *See, e.g.*, Appx36; Appx91; Appx153; Appx212; Appx267; Appx329 (citing Appx3816 and Appx3833). But each of the prior art references on which JSR and the Board relied concerns

modifications to Domains B and Z, as even JSR’s own expert acknowledged. *See, e.g.*, Appx6278-79; Appx7603 (Abrahmsén); Appx6258; Appx7606, Appx7611 (Linhult). By the Board’s own admission, “Linhult teaches making affinity chromatography columns with [domain] Z, Z(F30A), and additional mutated variants.” Appx33; Appx85; Appx146; Appx209; Appx261; Appx322. And “Abrahmsén, like Linhult, only exemplifies the cloning and expression of the Z-domain, which is a B-domain with a G29A mutation.”³ Appx34; Appx210.

The art’s undisputed, uniform focus on Domains B and Z is unsurprising.⁴ Domain B- and Domain Z-based SPA ligands, after all, had

³ In IPR2022-00041, IPR2022-0042, IPR2022-00044, and IPR2022-00045, the Board also tersely relied on Hober in its motivation findings. Appx90; Appx151; Appx266; Appx327. But Hober, which largely tracks and mirrors Linhult, identifies stability-enhancing mutations to Domain Z, not Domain C. *See* Appx3839-95; Appx7601 (Dr. Cramer stating that Hober’s work “was based on the B domain”). Hober does not describe any efforts to modify Domain C or present any data on modified Domain C proteins, and ultimately lauds the favorable characteristics of two Domain Z mutants. *See* Appx3877.

⁴ *See, e.g.*, Appx3816 (“To further increase the alkaline tolerance of SPA, we chose to work with Z, which is a small protein derived from the B domain of SPA.”); Appx3849 (“In one embodiment, the above described mutant protein is comprised of the amino acid sequence defined in SEQ ID NO 1 [Domain B] or 2 [Domain Z]”); Appx3896-3903 (opting to perform engineering on Domain B); Appx3911 (describing the MabSelect SuRe™ ligand, marketed

been demonstrated to have excellent binding capacity, and Domain Z had recently been incorporated into a commercially successful chromatography matrix. Appx7289-90. Domain B also was the best understood ligand, in part because its crystal structure was known. Appx7265 (citing Appx4152-61). Thus, if a POSA looking to design an SPA-based ligand with improved alkaline stability were to start with any particular domain, it would have been Domain B or the related Domain Z.

as an “alkali-stabilized ligand” and consisting of four Z domain units); Appx6191-96, Appx6209-14 (experimenting with Domain Z mutants); Appx7423-30 (studying the stability and unfolding of Domain B to, *inter alia*, better understand the IgG binding interface); Appx7431-38 (modifying Domain B to bind and elute IgG at higher pH values); Appx7439-46 (modifying Domain B, studying the stability of the modified SPA-IgG complex, and generating SPA variants with reduced binding affinities to allow elution at higher pH); Appx7447-54 (studying the impact of mutations to Domain Z on binding affinity); Appx7455-61 (modifying Domain Z for use in ion-exchange chromatography); Appx7462-73 (constructing destabilized mutants of Domain Z to allow elution at a pH as high as 4.5); Appx7474-80 (studying the affinity of various SPA derived proteins, including a monomer and oligomers of Domain Z); Appx7481-86 (designing affibodies, or small binding proteins, using combinatorial approaches based on Domain Z’s amino acid sequence); Appx7487-94 (producing a fusion protein with high affinity for the Fc fragment of IgG, said fusion protein comprising two mutated B domains); Appx7518 (“The present invention offers further benefits in that the Z domain can be stabilised to alkaline pH by replacement of asparagine residues. According to the present invention it is proposed to stabilise the native Z domain before the combinatorial library is prepared.”).

Of course, the POSA would know that Domain C existed. Domain C was a known domain in a naturally occurring protein. But the record is undisputed that *no one was working with Domain C* in the development of improved, modified ligands. *See* Appx6309-10 (Dr. Cramer unable to cite a single reference relating to research on Domain C). Nor does JSR's cited prior art single out Domain C for further development. *See* Appx7293-98. This is so despite JSR's expert, Dr. Cramer, performing a hindsight-driven prior art search for just such references. *See* Appx6309-10; Appx7592-93.

The Board's failure to consider whether and why the POSA would have nevertheless reviewed the sea of literature pointing to Domains B and Z, set it aside, and started over with the undeveloped Domain C ignores this Court's guidance. Whether an obviousness decision "is based on combining disclosures from multiple references, combining multiple embodiments from a single reference, or selecting from large lists of elements in a single reference, there must be a motivation to make the combination and a reasonable expectation that such a combination would be successful, otherwise a skilled artisan would not arrive at the claimed invention." *In re Stepan*, 868 F.3d 1342, 1345-46 n.1 (Fed. Cir. 2017). And "[w]hether a skilled artisan would be motivated to make a combination includes whether he would

select particular references in order to combine their elements.” *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1337 (Fed. Cir. 2016). Relatedly, an obviousness analysis must account for the prior art as a whole. “It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art.” *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965); *see also Impax Lab’ys Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018) (holding that a prior art reference did not render claims obvious because when viewing the reference “as a whole,” the reference was “not about intranasal formulations of zolmitriptan” which was “barely mentioned”).

Yet the Board did just that. Notwithstanding the host of literature—including JSR’s own references—pointing towards Domains B or Z as preferred starting points for designing an SPA-based ligand, the Board plucked out passing references to Domain C (or all five naturally occurring SPA domains) to supply the POSA’s motivation. *See* Appx31-35; Appx87-91; Appx148-52; Appx207-11; Appx263-67; Appx324-28. That the prior art regarding engineered forms of protein A focused exclusively on Domains B

and Z, and not Domain C, was a point of agreement between the parties' experts, and the Board made no findings to the contrary. *See* Appx6309-10; Appx7263-69; Appx7286-98.

2. JSR did not argue in its Petitions that the POSA would have selected Domain C as a lead compound, nor did it address *Otsuka* or the lead compound analysis. The closest it came during the proceedings was in two arguments, neither of which the Board relied on, and neither of which suffices to establish that the POSA would have selected Domain C for modification with a G29A mutation.

First, JSR argued that Linhult looked to Domain C, among other domains, when considering potential mutations to Domain Z. *See, e.g.*, Appx437-39; Appx1854. But Linhult focused on just one mutation from Domain C—N23T—and did not suggest adopting Domain C wholesale, either as a target sequence or as a starting point for developing a new one. *See* Appx3820, Appx3822; Appx7276-80. Nor did Linhult teach Domain C as a starting point for designing an SPA-based ligand. But *See* Appx2052-56; Appx7293-98. JSR did not explain, nor could it, how the limited use of Domain C in Linhult would have given the POSA reason to pursue the C(G29A) sequence specifically, only to use certain “C-domain-specific

residues.” Appx437. Nor did JSR argue in its Petition that Linhult—which concededly studied “synthetic Z domain (*i.e.*, B(G29A)-based ligands having additional mutations”)—would have motivated the POSA to use Domain C as a lead compound. Appx437.

Second, JSR pointed to the teachings of an additional reference, Berg (Appx4162-71), not relied upon by the Board, that mentions Domain C as one of two “preferabl[e]” ligands, albeit with no explication. Appx4166 (“In a specific embodiment, the ligands of the present separation matrix comprise a monomer, dimer or multimer of Protein A domains. Thus, the ligands may comprise one or more of Domain A, B, C, D and E, preferably Domain B and/or Domain C.”). But Berg does not supply the requisite teaching to select Domain C as a lead compound. At the outset, Berg, a patent publication from Cytiva’s predecessor, is not prior art because the relied-upon disclosures are not inventive work “by others.” *In re Katz*, 687 F.2d 450, 454 (C.C.P.A. 1982); *see, e.g.*, Appx1592-95 (explaining why Berg does not qualify as prior art); Appx2151-55 (same). Regardless, the POSA would not have plucked Berg and its fleeting reference to Domain C from the veritable ocean of prior art focused on Domains B and Z, nor did JSR explain why, without hindsight reasoning, the POSA would have done so. *See WBIP*,

829 F.3d at 1337 (requiring courts to ask “whether th[e] skilled artisan would have plucked one reference out of the sea of prior art . . . and combined it with [a known element] to address some need present in the field”). And even if the POSA did, it was undisputed that Berg focuses on the properties of particles to which ligands are immobilized and not the ligands themselves. Appx7290-93; Appx6295-96 (Dr. Cramer agreeing that Berg is directed to engineering the chromatography resin, or solid support, rather than protein A ligands). Berg’s reference to Domain C is surrounded by references to Domains B and Z, including multiple citations to Hober, which itself explores various mutations to Domain Z. *See* Appx4166. As a matter of law, Berg fails to supply the motivation to select Domain C as a lead compound over other SPA domains, such as Domains B and Z.⁵

⁵ Because Berg is insufficient to establish a motivation to use Domain C as a lead compound and JSR did not argue otherwise in its Petitions, this Court can and should simply reverse the Board’s unpatentability decisions without remanding for the Board to consider Berg. But to the extent the Court determines that a remand on this basis is necessary, only the petitions relying on Berg (IPR2022-00043, IPR2022-00044, and IPR2022-00045) should be sent back for that limited purpose.

C. The Board’s Recourse to *KSR* Does Not Justify Short-Circuiting the Lead Compound Analysis.

Perhaps cognizant of these deficiencies, JSR succeeded in urging the Board to forgo a lead compound analysis altogether and instead find that a G29A modification to *any one* of the five SPA domains was among a finite number of predictable solutions, and thus obvious under *KSR*. Adopting *KSR*’s framework, the Board concluded “it is fair to say that there were “a finite number of identified, predictable solutions” to the problem of finding’ a SPA IgG binding domain that is resistant to protein degradation by mutating the glycine at position 29 for an alanine and this is a ‘product not of innovation but of ordinary skill and common sense.” Appx36-37; Appx212-13 (quoting *Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012)); *see also* Appx92; Appx153-54; Appx268-69; Appx329-30 (similar).

In this analysis, the Board did not consider “whether a chemist of ordinary skill would have selected the asserted prior art compounds as lead compounds.” *Otsuka*, 678 F.3d at 1291. Instead, the Board started from the proposition that SPA’s IgG binding domains “comprise a short list of 5 members: E, D, A, B, or C,” and those domains “share many structural similarities.” Appx36-37; Appx91-92; Appx153; Appx212-13; Appx267-68;

Appx329. The Board then reasoned that Linhult and Abrahmsén teach mutating “the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which supports the obviousness of incorporating the mutation into any IgG binding domain that has the Asn-Gly dipeptide.” Appx37; Appx213; *see* Appx91; Appx154; Appx269; Appx329 (similar). But this is the *second* step of the lead compound analysis—“whether the prior art would have supplied one of ordinary skill in the art with a reason or motivation to modify a lead compound to make the claimed compound,” *Otsuka*, 678 F.3d at 1292—applied *en masse* to all of the naturally occurring SPA domains mentioned in Linhult and Abrahmsén, without any consideration of whether those would have been the POSA’s starting points.

That approach improperly risks—and here, fell victim to—reliance on “*ex post* reasoning” against which this Court cautioned in *Otsuka*. *Id.* (citing *KSR*). As the Court recognized, structural similarity between a prior-art compound and a claimed compound often can be found; the question for obviousness is whether the POSA would have had reason to identify a connection between those compounds so as to make the same new compound that the inventor did. *See id.*

Contrary to the Board’s holding, *KSR* cannot substitute for the first step of the lead-compound analysis because in this case, as is commonplace in the chemical arts, the number of options available to the POSA would have been anything but “finite” or “predictable.” In *KSR*, the Supreme Court recognized that in the absence of an express teaching in the art, courts that rigidly applied a motivation-to-combine analysis would sometimes anomalously uphold patent claims that are directed to straightforward design choices that the POSA would have considered—in *KSR*, the decision to locate an electronic sensor at the fixed pivot point of a pedal rather than elsewhere in the pedal structure. *KSR*, 550 U.S. at 410-11. Underlying the Court’s analysis was a recognition that in such circumstances, the number of solutions available to the POSA was relatively constrained—“finite,” as the Court put it—and the consequences of those solutions were “predictable.” *See id.* at 421.

Here, however, the Board did not consider whether the universe of solutions available to the POSA, or even a subset of those solutions that the POSA may have had reason to focus on, were “finite” or “predictable.” Instead, the Board asked whether the naturally occurring SPA ligands *disclosed in JSR’s chosen references* constituted a finite set of options, and

whether the change proposed in those references—the G29A substitution—had predictable consequences for the ligand’s stability. Appx31-42; Appx87-96; Appx148-59; Appx207-18; Appx263-73; Appx324-35. But the Board’s findings on these points do nothing to address *Otsuka*’s concern of avoiding hindsight reasoning. Undisputedly, Domains A, B, C, D, and E are not the only possible starting points for creation of a stable ligand useful in the claimed compositions and methods. *See* Appx2974-78 (Dr. Cramer discussing the field’s acceptance of Domain Z as an alkaline-stabilized SPA ligand); Appx7264-65 (Dr. Bracewell discussing the prevalence of Domain Z in SPA affinity chromatography research); *see also* Appx6268 (Dr. Cramer acknowledging Linhult’s preferred Z(N23T) mutant as a plausible starting point for further development of a stable ligand). On the contrary, the undisputed record establishes that *most* activity in the field was based around modifications to Domain Z (which itself already possessed good alkaline stability). *See supra* p. 27 n. 4; *see also* Appx6309-10 (Dr. Cramer unable to recall or cite any experimental data involving mutated versions of Domain C). JSR’s own references, moreover, include other improved ligands that contain promising sequence modifications, and those very references teach that many of these ligands are improvements over Domain Z (not the

natural ligands). Appx3820 (identifying Z(N23T) as “the most promising candidate as [sic] ligand in affinity purification of IgG”); Appx3877 (“Both Z(N23T/N3A/N6D)dimer-Cys and Z(N23T/N3A)dimer-Cys showed improved stability against alkaline conditions compared to the originally produced Z(N23T)dimer-Cys.”).

Nothing in the Board’s opinions or this Court’s case law justifies the Board’s decision to eschew this broad universe of possibilities and look solely to the five natural ligands, optionally modified with the G29A mutation (and only the G29A mutation). It is true, and a truism, that the set of five natural ligands is “finite,” but the set of options available to the POSA was vastly broader. *See, e.g.*, Appx7320-21 (Dr. Bracewell describing a subset of possible options). Nothing in the Board’s decision or this Court’s precedents justifies the use of the *KSR* obvious-to-try framework to winnow that broader universe solely to JSR’s preferred starting points. That winnowing should have been the role of the lead-compound analysis that the Board skipped.

KSR also cannot displace the necessary lead compound analysis because the field of protein engineering is anything but “predictable.” *See Abbott Lab’ys v. Sandoz, Inc.*, 544 F.3d 1341, 1352 (Fed. Cir. 2008) (“The

Court in *KSR* did not create a presumption that all experimentation in fields where there is already a background of useful knowledge is ‘obvious to try,’ without considering the nature of the science or technology.”); *Eisai Co. v. Dr. Reddy’s Lab’ys, Ltd.*, 533 F.3d 1353, 1359 (Fed. Cir. 2008) (“To the extent an art is unpredictable, as the chemical arts often are, *KSR*’s focus on . . . ‘identified, predictable solutions’ may present a difficult hurdle because potential solutions are less likely to be genuinely predictable.”); *see also In re Schechter*, 205 F.2d 185, 191 (C.C.P.A. 1953) (isomers and homologs of compounds found in the prior art nonetheless patentable because “there is a considerable degree of unpredictability in the insecticide field”). The parties’ experts agreed on this score. *See* Appx6260 (Dr. Cramer stating that determining the impacts of amino acid substitutions on antibody binding “would require a detailed analysis”); Appx6261 (Dr. Cramer opining that “it would be very difficult to predict . . . a priori” what effect a mutation would have on antibody binding); Appx6266 (Dr. Cramer stating “[i]t would be difficult to predict” whether a single amino acid change to one SPA domain would have the same effect on antibody binding if that change were made to a different SPA domain); Appx7263-64 (“[P]redicting *ex ante* whether an amino acid substitution would impact SPA’s characteristics as a ligand for

use in affinity chromatography [as of 2006] was still largely a guessing game requiring extensive experimentation.”). And the patent examiner’s statements during prosecution of the ’765 patent buttress this fact: “Protein chemistry is probably one of the most unpredictable areas of biotechnology, and “the effects of sequence dissimilarities upon protein structure and function cannot be predicted.” Appx4326.

The impact of a G29A mutation on Domain Z’s Fab-binding ability amply demonstrates the unpredictability of even single point mutations. Introducing a G29A mutation to Domain B (*i.e.*, creating Domain Z) was found to improve its alkaline stability and not “interfere much with binding to Fc.” Appx7375. However, that same mutation was found to result in “only little or no [Fab] binding activity,” illustrating how a single amino-acid mutation may have little impact on some properties even as it has a drastic effect on others. Appx7375; *see also* Appx7476 (reaching the “most obvious” conclusion that the Z domain “hardly bind[s]” to Fab); Appx5920 (“The Z-domain . . . was shown to have negligible binding to the antibody variable region.”); Appx5961 (“It is interesting to note that it only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine

to an alanine . . . while inactivation of tyrosine residues by iodination eliminates Fc binding in SPA . . .”).

The Board’s findings that the POSA would reasonably expect that a G29A mutation in any one of the five naturally occurring protein A domains would increase the domain’s alkaline stability while maintaining binding capacity for IgG, *see* Appx38-42; Appx93-97; Appx155-59; Appx214-18; Appx269-73; Appx331-35, do not alter this analysis. The Board did not—and could not—find that the POSA could predict other properties of an SPA-based ligand with a G29A mutation, reinforcing the unpredictability of protein engineering. For instance, as already explained, to the extent the POSA could predict anything about the Fab binding properties of an SPA-based ligand with a G29A mutation, the POSA would have predicted that such a ligand would lack the ability to bind Fab fragments. *See supra* pp. 10, 39-40; Appx7266-68, Appx7313-15.

* * *

In short, the Board committed legal error when it leapfrogged the required analysis that the POSA would have selected Domain C as a starting point for designing an SPA-based affinity ligand notwithstanding the art’s

preference for alternative solutions available to the POSA in a larger and notoriously unpredictable field.

II. The Board Erred in Ruling that the Dependent Fab Binding Claims are Unpatentable.

Even if the Board were correct in its decision to find the independent claims unpatentable under a *KSR*-style obviousness analysis, it then erred in extending this reasoning to the inventors' discovery that their novel C(G29A) ligand could bind to the Fab part of an antibody, which is reflected in claims 4 and 17 of the '765 patent.

Hornbook law requires that in assessing the obviousness of the claims, the Board must assess whether the POSA would have a motivation or reason to combine elements from the prior art into the claimed invention with a reasonable expectation of success. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007); *Orexo AB v. Actavis Elizabeth LLC*, 903 F.3d 1265, 1271 (Fed. Cir. 2018); *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Pat. Litig.*, 676 F.3d 1063, 1068-69 (Fed. Cir. 2012). The Board also was obligated to account for objective indicia of nonobviousness, including any evidence of the invention's unexpected properties. *In re Cyclobenzaprine*, 676 F.3d at 1075-76, 1079. With respect to the claims of the '765 patent, however, the Board expressly *refused* to consider evidence of the

surprising Fab-binding capacity of the claimed ligand, declining to entertain Cytiva's arguments that the unforeseen nature of this feature both undermined any reasonable expectation of success for composition claims requiring Fab binding and constituted an unexpected result probative of nonobviousness. *See, e.g.*, Appx44-49.

Here, the record reflects that the C(G29A) ligand exhibits Fab binding, *see, e.g.*, Appx367; the prior-art Domain Z (*i.e.*, a B(G29A) ligand) does *not* exhibit Fab binding, *see, e.g.*, Appx5920; Appx5961; Appx7375, Appx7476; and the POSA would not have expected the C(G29A) ligand to exhibit such binding, *see supra* pp. 10, 39-40. As a matter of law, the Board's recourse to inherency cannot support a finding that claims requiring this unexpected property would have been obvious, particularly where those claims identify particular ligands that exhibit the property from a broader group of putatively obvious-to-try ligands that do not. In addition, the Board's failure to consider evidence of unexpected properties fatally undermines its obviousness holding.

A. The Board Cannot Rely on Inherency to Avoid Consideration of Whether the POSA Would Have Had a Reasonable Expectation of Success.

The Board excused its failure to perform these required steps of the obviousness analysis by pointing to the alleged inherency of the claimed ligand's Fab-binding capability. *See* Appx44-48; Appx220-24. This Court, however, has warned that "the use of inherency, a doctrine originally rooted in anticipation, must be carefully circumscribed in the context of obviousness." *Par Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1195 (Fed. Cir. 2014). Far from treading carefully, the Board's decision reflects an aggressive extension of inherency, and one that runs roughshod over many decades of precedent.

When an obviousness challenge is premised on the combination of disclosures from various prior art references, it is well established that § 103 requires consideration of whether the POSA in fact would have (i) been motivated to combine the teachings of those references and (ii) had a reasonable expectation of success in doing so. This Court and its predecessor have echoed that rule for decades. *See, e.g., Orexo*, 903 F.3d at 1271; *InTouch Techs., Inc. v. VGO Commc'ns, Inc.*, 751 F.3d 1327, 1347 (Fed. Cir.

2014); *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991); *In re Rinehart*, 531 F.2d 1048, 1053-54 (C.C.P.A. 1976).

The issue before the Board here was whether the POSA would have had a reasonable expectation of combining the disclosures of the prior art to achieve a C(G29A) ligand “capable of binding to the Fab part of an antibody.” That is what claims 4 and 17 of the ’765 patent recite, and “[t]he reasonable expectation of success requirement refers to the likelihood of success in combining references to meet *the limitations of the claimed invention*.” *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1367 (Fed. Cir. 2016) (emphasis added); *see also, e.g., Allergan*, 754 F.3d at 966. Such evidence of a reasonable expectation of success was entirely lacking. On the contrary, the extensive and undisputed evidence below established that protein engineering is a notoriously unpredictable art, particularly when it comes to how a ligand binds to antibodies.⁶ Even worse for JSR, the

⁶ See Appx6260 (Dr. Cramer agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability and stating that determining the impacts of amino acid substitutions on antibody binding “would require a detailed analysis”); Appx6261 (Dr. Cramer opining that “it would be very difficult to predict . . . a priori” what effect a mutation would have on antibody binding); Appx6266 (Dr. Cramer stating “[i]t would be difficult to predict” whether a single amino acid change to one SPA domain would have the same effect on antibody binding if that change were made to

evidence also demonstrated that, to the extent the POSA could make any predictions about the results of a G29A mutation in the amino acid sequence of Domain C, she would have assumed such a modification would have *removed* Domain C's ability to bind Fab. Appx7303-07, Appx7314-18 (Dr. Bracewell explaining why the POSA would have expected, if anything, a C(G29A) ligand to lose Fab binding). That is precisely what occurred when the same change was made to the homologous Domain B sequence to create Domain Z. Appx7368-77 (Jansson demonstrating that "Domain B clearly binds Fab whereas the very similar Domain Z shows only little or no binding activity"); Appx5961 ("[T]he engineered Z domain binds Fc but lacks the ability to bind Fab."); Appx7479 ("[T]he A, D and/or E regions contain a structural motif, which is not present in Z, and which is necessary for Fab-binding."); Appx5920 ("The Z-domain . . . was shown to have negligible binding to the antibody variable region."). In other words, far from having

a different SPA domain); Appx7266-69 (Dr. Bracewell agreeing with Dr. Cramer regarding the unpredictability of engineering protein A to improve its function as an affinity ligand); Appx7361 (patent examiner stating that "[p]rotein engineering is probably one of the most unpredictable areas of biotechnology," and that "the effects of sequence dissimilarities upon protein structure and function cannot be predicted.").

an expectation of success with respect to claims 4 and 17, the POSA would have had an expectation of *failure*.

The Board made findings in Cytiva's favor on each of these points with respect to claims 4 and 17 of the '142 patent and claims 11 and 29 of the '007 patent—*process* claims requiring Fab binding. *E.g.*, Appx104-05; Appx166. In those IPRs, the Board held JSR to the correct standard: “the evidence needs to show a reasonable expectation that a mutated SPA binds Fab.” Appx163; *see* Appx102. Applying this standard, the Board correctly found that “each of Linhult, Abrahmsén, or Hober is silent with respect to Fab binding to a mutated SPA domain.” Appx102-03; Appx164. Further, the Board correctly found that Jansson's Figure 3 “shows that the G29A mutation results in a loss of Fab binding ability,” and addressed the art's teachings that “the site responsible for Fab binding is structurally separate from the domain surface that mediates Fcy binding.” Appx104; Appx165. Finally, the Board reasoned that establishing that the G29A mutation would not “interfere with IgG binding says nothing about the ability of a mutated SPA domain to bind Fab.” Appx165; *see* Appx104. Putting these findings together, the Board correctly concluded that the prior art supported Cytiva's position that Fab binding in the C(G29A) ligand was unexpected, and that

JSR “has not established by a preponderance of the evidence of record that the process of isolating [a] Fab target using a mutated SPA domain C ligand as required by claims 11 and 29 would have been obvious based on the combined teachings of Linhult, Abrahmsén, and Hober.” Appx166; *see* Appx104-05 (same for claims 4 and 17 of the ’142 patent).

These findings put the Board’s holding of obviousness with respect to the *composition* claims reciting Fab binding—claims 4 and 17 of the ’765 patent—into sharp relief. As to those claims, the Board relied on the doctrine of inherency to improperly reframe this expectation question away from the claim limitations. Dismissing Fab binding as a mere inherent property of the claimed C(G29A) ligand, the Board concluded that JSR had shown there would be “a reasonable expectation that making a G29A mutation in any one of the SPA domains would result in a structure that retains the ability to bind IgG” and that this was sufficient. Appx48; Appx224 (emphasis removed). But “binding IgG” does not necessarily mean binding the Fab part of that or any other antibody, and the Board was not entitled to shift the goalposts of the expectation analysis by substituting IgG binding for the express language of the claims.

Courts have long maintained that, while the doctrine of inherency may be used to supply a missing claim limitation not expressly disclosed in the prior art, it cannot absolve a challenger of its obligation to demonstrate a reasonable expectation of success. *See, e.g., In re Rinehart*, 531 F.2d at 1053-54. That is particularly important where the properties at issue would have been unexpected: “All properties of a composition are inherent in that composition, but unexpected properties may cause what may appear to be an obvious composition to be nonobvious.” *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. DE C.V.*, 865 F.3d 1348, 1354-55 (Fed. Cir. 2017) (“What is important regarding properties that may be inherent, but unknown, is whether they are unexpected.”). “That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown.” *In re Spormann*, 363 F.2d 444, 448 (C.C.P.A. 1966).

The *Rinehart* decision is instructive. The claims at issue recited methods for the commercial-scale production of polyesters. 531 F.2d at 1049. This Court’s predecessor reversed a decision from the Patent and Trademark Office Board of Appeals affirming an examiner’s rejection under § 103. *Id.* at 1054. The *Rinehart* court explained:

The tribunals below did not meet the requirement of establishing some predictability of success in any attempt to combine elements

of the refer[en]ce processes in a commercial scale operation. As in *In re Naylor*, 369 F.2d 765, 54 CCPA 902 (1966), we find nothing in the record which would lead one of ordinary skill to anticipate successful production on a commercial scale from a combination of such elements, without increase in glycol-acid ratio. The record in fact reflects the contrary. The view that success would have been ‘inherent’ cannot, in this case, substitute for a showing of reasonable expectation of success. Inherency and obviousness are entirely different concepts.

Id. at 1053-54. The Board should have engaged in a similar analysis here.

There was “nothing in the record which would lead one of ordinary skill to anticipate successful” Fab binding with a C(G29A) ligand, and “[t]he record in fact reflects the contrary.” The Board’s “view that [Fab binding capability] would have been ‘inherent’ cannot . . . substitute for a showing of reasonable expectation of success.”

The Board’s citations to *In re Pearson*, 494 F.2d 1399 (C.C.P.A. 1974) and *Persion Pharms. LLC v. Alvogen Malta Operations Ltd.*, 945 F.3d 1184 (Fed. Cir. 2019), do not alter that conclusion. Appx46; Appx222. Certainly, this Court has observed on occasion that claim terms that “merely set forth the intended use for, or a property inherent in, an otherwise old composition” may not “differentiate the claimed composition from those known to the prior art.” *Pearson*, 494 F.2d at 1403. But such statements did not reverse long-standing precedent nor write the reasonable expectation of success

requirement out of § 103. Rather, they arose in the particular factual circumstances presented by the cases then before the Court, and those circumstances very much matter.

Inherency, of course, is a doctrine born from anticipation, *Par Pharm.*, 773 F.3d at 1195, and in that original context its application is relatively straightforward. If a claimed composition is anticipated, it is by definition not new; it existed or was disclosed, in complete form, in the prior art, and it is axiomatic that it therefore cannot be patented. The inherency doctrine forbids a patentee from manufacturing novelty by pointing to an inherent feature of its claimed composition—regardless of whether the feature had previously been recognized, that composition already existed in the prior art and cannot be patented again. *See Atlas Powder Co. v. Ireco, Inc.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999). For these reasons, this Court has long recognized that a prior art reference may inherently anticipate a claimed invention without expressly disclosing a feature of the claimed invention if that missing characteristic is necessarily present. *See Trintec Indus., Inc. v. Top-U.S.A. Corp.*, 295 F.3d 1292, 1295 (Fed. Cir. 2002). “The mere fact that a certain thing *may* result from a given set of circumstances is not

sufficient.” *Continental Can Co. USA, Inc. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991) (emphasis in original) (citation omitted).

In the obviousness context, however, the inquiry is necessarily murkier because the composition definitionally did *not* exist in the prior art (if it did, it would be anticipated). The above logic therefore does not hold. The patent system has grappled with two competing interests, and permitted recourse to inherency only in narrow circumstances in the context of obviousness. On the one hand, where the teachings of the art would have pointed the POSA in the direction of a claimed composition for reasons other than the inherent property, a patentee’s claims drawn to undisclosed, inherent features of such composition may add little or no value for the public—the art already instructs the POSA to make that particular composition and, in doing so, the POSA will come to possess the advantageous features therein. The would-be patentee has not disclosed any “new and useful advance[] in technology” and has not upheld its end of the patent “bargain.” *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 63 (1998) (“[T]he patent system represents a carefully crafted bargain that encourages both the creation and the public disclosure of new and useful advances in technology, in return for an exclusive monopoly for a limited period of time.”).

On the other hand, however, when an inventor recognizes that by making a particular new composition, a hitherto unknown and unexpected property emerges, she has thereby made the type of discovery that the patent system is intended to incentivize. And, rightly, no case that JSR or the Board cited suggests that this discovery becomes unpatentable just because the prior art teaches in the general direction of an entire *class or category* of compositions to which that particular composition belongs, all of which may have *different* inherent properties. In that scenario, a patentee's identification of an unexpected and advantageous property of a *particular* composition within the class adds to the public's knowledge. Granting a patent on such discovery affords the public its benefit of the patent bargain.

That is precisely the scenario presented by this case. The Board's obviousness conclusion was broad: it found that the prior art "suggest[ed] making the G29A mutation in *any one* of the SPA IgG binding domains E, D, A, B, or C." *E.g.*, Appx35. Nothing in the Board's reasoning pointed the POSA to Domain C in preference to A, B, D, or E, and in fact the Board expressly rejected the premise that it needed to find that Domain C would constitute a "lead." *See, e.g.*, Appx35-38. The very same reasoning points equally towards a B(G29A) ligand that, as the art establishes, *lacks* the

capability of binding to Fab. *See supra* pp. 10, 39-40. And it also points equally towards unclaimed A(G29A), D(G29A), and E(G29A) ligands, the Fab-binding capabilities of which were—as far as Cytiva is aware, and as far as the record reflects—entirely unknown. The claims at issue are comparatively narrow and directed only to C(G29A) ligands and their unexpected and beneficial Fab-binding capability. Cytiva’s discovery of that property and disclosure of the same provided the public a benefit meriting patent protection.

Neither the authority relied upon by the Board nor any of this Court’s recent decisions compel a different result. *In re Pearson*, 494 F.2d 1399 (C.C.P.A. 1974) resolved a question of anticipation, not obviousness. *Id.* at 1402 (“[T]he board interpreted the examiner’s reasoning, accurately we think, as setting forth as one ground for the rejection that the claims did not define novel subject matter.”). Moreover, the court took pains to emphasize the limits of its inherency analysis, warning that where there exists some difference between the claimed composition and the composition(s) described in the prior art (as would be the case in an obviousness analysis), claim terms describing some inherent property of the new, claimed composition can render that composition nonobvious. *Id.* at 1403 (“We do not mean to imply

that terms which recite the intended use or a property of a composition can never be used to distinguish a new from an old composition. However, . . . such terms must define, indirectly at least, some characteristic not found in the old composition.”). The sweeping inherency rule the Board now proposes therefore finds no support in *Pearson*. *Pearson* is inconsistent with such a rule, and emphasized the opposite.

Persion Pharmaceuticals is at least an obviousness case, but it presents the very different obviousness scenario discussed above—one in which the prior art taught directly towards a particular composition (or, more precisely, towards the use of a particular formulation in a particular patient population). The asserted claims recited methods of treating pain in patients with mild or moderate hepatic impairment by administering an extended-release formulation of hydrocodone. *Persion Pharms.*, 945 F.3d at 1186-87. Certain claims also recited pharmacokinetic results to be achieved through that administration. *Id.* The prior art disclosed the *same* extended-release formulation as well as various methods of treating pain in patients with mild or moderate hepatic impairment using extended-release and immediate-release hydrocodone products. *Id.* at 1187-88. Rather than rendering a whole class or category of methods obvious, the combination of

art before the district court gave the POSA reason to practice the specific claimed method. In such circumstances, the court's invocation of inherency to find the pharmacokinetic limitations were met was consistent with § 103, this Court's decisions interpreting that section, and the policy concerns animating it.

Hospira, Inc. v. Fresenius Kabi USA, LLC, 946 F.3d 1322 (Fed. Cir. 2020)—a decision not cited by the Board or JSR—tracks *Persion* and does not excuse JSR of its obligation to prove a reasonable expectation of success. The asserted claims in *Hospira* were directed to ready-to-use formulations of a prior art compound that, when stored in a glass container, exhibit “no more than about 2% decrease in the concentration of” the compound. *Id.* at 1326. The prior art disclosed a specific ready-to-use formulation but did not expressly teach the “about 2%” degradation limitation. *Id.* at 1327. The district court relied on inherency to fill the gap. That conclusion again neatly fits within the contours of the rule described above: the prior art renders a particular formulation obvious and the patentee's claims drawn to the inherent features of that formulation add little or no value for the public. What is more, this Court did not excuse the patent challenger of its obligation to demonstrate a reasonable expectation of success in making the

claimed invention. To the contrary, this Court stressed that requirement before noting that “the parties [did] not dispute that [the patent challenger] met its burden of proof on that issue.” *Id.* at 1331. “Thus, the only dispute [wa]s whether the district court’s inherency analysis was correct.” *Id.* The same cannot be said here.

In other recent decisions, this Court has emphasized the importance of the reasonable expectation of success inquiry in the face of inherency arguments. In *Par Pharmaceutical*, for example, the Court warned that “the concept of inherency must be limited when applied to obviousness” and cautioned against an overreading of an earlier case in which it was stated that “an obvious formulation cannot become nonobvious simply by administering it to a patient and claiming the resulting serum concentrations.” 773 F.3d at 1195 (quoting *Santarus, Inc. v. Par Pharm., Inc.*, 694 F.3d 1344, 1354 (Fed. Cir. 2012)). While that statement was correct, the *Par* court emphasized that in this earlier case “[i]mportantly . . . neither party disputed that the blood serum concentrations claimed . . . were expected in light of the dosages disclosed in the prior art.” *Id.*

Similarly, in *Millennium Pharmaceuticals, Inc. v. Sandoz Inc.*, 862 F.3d 1356, 1367 (Fed. Cir. 2017), the Court stressed that “[t]he mere fact that

a certain thing may result from a given set of circumstances is not sufficient” to render the result inherent. “[T]he inventor’s own path itself never leads to a conclusion of obviousness; that is hindsight. What matters is the path that the person of ordinary skill in the art would have followed, as evidenced by the pertinent prior art.” *Id.* (quoting *Otsuka*, 678 F.3d at 1296). Because “[n]o expert testified that they foresaw, or expected” that the lyophilization of a prior art compound and mannitol—a known bulking agent—would result in the new, claimed compound, the inherency doctrine was inapplicable. *Id.*

The Board’s reflexive move to set aside the required reasonable expectation of success analysis at the mere mention of inherency was an unwarranted extension of this Court’s inherency decisions and resulted in legal error. The Board was required to assess whether the POSA would have a reasonable expectation of success at combining the disclosures in the prior art to achieve the claimed invention—here, a C(G29A) ligand “capable of binding to the Fab part of an antibody.” Because the evidence of record shows just the opposite, the Board’s decision must be reversed.

B. The Board Cannot Rely on Inherency to Avoid Consideration of Objective Indicia of Nonobviousness.

Objective indicia of non-obviousness must be considered if present. *In re Cyclobenzaprine*, 676 F.3d at 1075-76, 1079; *Knoll Pharm. Co. v. Teva*

Pharms. USA, Inc., 367 F.3d 1381, 1385 (Fed. Cir. 2004). Such evidence can be instrumental in “guard[ing] against slipping into use of hindsight” and “the temptation to read into the prior art the teachings of the invention at issue.” *Apple Inc. v. Samsung Elecs. Co.*, 839 F.3d 1034, 1052 (Fed. Cir. 2016) (en banc) (quoting *Graham v. John Deere Co.*, 383 U.S. 1, 36 (1966)); *see also, e.g., WBIP*, 829 F.3d at 1328 (“[O]bjective indicia of non-obviousness play an important role as a guard against the statutorily proscribed hindsight reasoning in the obviousness analysis.”). “Indeed, evidence of secondary considerations may often be the most probative and cogent evidence in the record,” and “may often establish that an invention appearing to have been obvious in light of the prior art was not.” *Apple*, 839 F.3d at 1052 (quoting *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538-39 (Fed. Cir. 1983)).

For more than 100 years, courts have recognized that the new and unexpected properties of an invention may function as such indicia of nonobviousness. The Supreme Court explained this principle succinctly in *Loom Co. v. Higgins*, 105 U.S. 580 (1881): “It may be laid down as a general rule, though perhaps not an invariable one, that if a new combination and arrangement of known elements produce a new and beneficial result, never

attained before, it is evidence of invention.” *Id.* at 591. A long line of decisions from this Court and its predecessor have reaffirmed that tenet repeatedly over the intervening years and made clear that it does not matter whether the unexpected feature was inherent in the invention. *See, e.g., Honeywell Int’l*, 865 F.3d at 1354-55 (“What is important regarding properties that may be inherent, but unknown, is whether they are unexpected. All properties of a composition are inherent in that composition, but unexpected properties may cause what may appear to be an obvious composition to be nonobvious.”); *Allergan, Inc. v. Sandoz Inc.*, 796 F.3d 1293, 1307 (Fed. Cir. 2015) (“The unexpected properties of the claimed formulation, even if inherent in that formulation, differ in kind from the prior art, thereby supporting a conclusion of nonobviousness.”); *Knoll Pharm.*, 367 F.3d at 1385 (finding that post-filing work performed to establish the unexpected, inherent property of a claimed invention was properly considered in assessing obviousness); *In re Khelghatian*, 364 F.2d 870, 876 (C.C.P.A. 1966) (same); *In re Naylor*, 369 F.2d 765, 767-68 (C.C.P.A. 1966) (“We cannot ignore the particular product unexpectedly produced by the claimed process, as the Patent Office apparently has done, in determining whether the claimed subject matter as a whole is obvious.”); *In re*

Spormann, 363 F.2d at 448 (“[T]he inherency of an advantage and its obviousness are entirely different questions. That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown.”).

The Board ignored this precedent to reach its obviousness conclusion in the face of evidence that the ability of the claimed ligand to bind the Fab part of an antibody was wholly unexpected and contrary to the teachings of the prior art. *See supra* pp. 10, 39-40, 45-47. Seemingly conflating the question of unexpected results with the separate consideration of whether the POSA would have had a reasonable expectation of success,⁷ the Board stated that “[t]he prior art does not need to recognize that Domain C retains the ability to bind Fab fragments after a G29A mutation,” and found that “there is a reasonable expectation that making a G29A mutation in *any one* of the SPA domains results in a product that binds at least IgG.” Appx48; Appx224. But the Board was required to assess any unexpected properties of the invention regardless of whether there existed a reasonable expectation

⁷ The answer to that latter inquiry also is “no.” As discussed above, the POSA would have had no reasonable expectation that the ligand of claims 4 and 17 of the ’765 patent would be capable of successfully binding the Fab part of an antibody. *See supra* Section II.A.

of success; these are two distinct inquiries. Nor could the Board skip this step of the obviousness analysis by resort to inherency. *See, e.g., Honeywell Int'l*, 865 F.3d at 1354-55; *Allergan*, 796 F.3d at 1307; *Kloster Speedsteel AB v. Crucible Inc.*, 793 F.2d 1565, 1576 (Fed. Cir. 1986), *as amended on reh'g* (Aug. 15, 1986) (“That argument is unpersuasive when confronted by [defendant’s] failure to establish at trial that that inherency would have been obvious to those skilled in the art when the invention of claim 4 was made. Inherency and obviousness are distinct concepts.”). The Fab-binding capability of a C(G29A) ligand was wholly unexpected and contrary to the teachings of the prior art, which warned of the dire Fab-binding consequences of a G29A mutation in Domain B. *See supra* pp. 10, 39-40, 45-47. As in *Allergan*, “[t]he unexpected properties of the claimed [ligand], even if inherent in that [ligand], differ in kind from the prior art, thereby supporting a conclusion of nonobviousness.” 796 F.3d at 1307.

The Board’s dereliction of its duty to consider objective indicia of nonobviousness here was error, and its reliance on *Persion Pharmaceuticals* to justify that failure was misplaced. While this Court did acknowledge in *Persion* that “[i]nherency may supply a missing claim limitation in an obviousness analysis,” it nowhere suggested that “inherency” could be used

to ignore or discount the existence of unexpected properties as objective evidence of nonobviousness. *Persion*, 945 F.3d at 1190 (quoting *Par*, 773 F.3d at 1194-95). To the contrary, *Persion* expressly addressed such indicia, noting that “the district court . . . considered [patentee’s] evidence of objective indicia,” including “testimony of the inventors regarding *unexpected results*.” *Id.* at 1194 (emphasis added).

CONCLUSION

For the foregoing reasons, Cytiva requests that the Court reverse the Board’s Final Written Decisions as to claims 1-7, 10-20, and 23-26 of the ’765 patent; claims 1-3, 5-7, 10-16, 18-20, and 23-30 of the ’142 patent; and claims 1-10, 12-14, 16-28, 30-32, and 34-37 of the ’007 patent.

Respectfully submitted,

/s/ David M. Krinsky

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OCTOBER 27, 2023

CERTIFICATE OF SERVICE

I, David M. Krinsky, counsel for appellant and a member of the Bar of this Court, certify that, on October 27, 2023, a copy of the attached Opening Brief of Appellant Cytiva Bioprocess R&D AB was filed with the Clerk and served on the parties through the Court's electronic filing system. I further certify that all parties required to be served have been served.

OCTOBER 27, 2023

/s/ David M. Krinsky
DAVID M. KRINSKY

**CERTIFICATE OF COMPLIANCE WITH
TYPEFACE LIMITATION AND WORD COUNT**

I, David M. Krinsky, counsel for appellant and a member of the Bar of this Court, certify, pursuant to Federal Rule of Appellate Procedure 32(a)(7)(B) and Federal Circuit Rule 32(b), that the attached Opening Brief of Appellant Cytiva Bioprocess R&D AB is proportionately spaced, has a typeface of 14 points or more, and contains 13,014 words, excluding the parts of the Brief exempted by Federal Rule of Appellate Procedure 32(f) and Federal Circuit Rule 32(b).

OCTOBER 27, 2023

/s/ David M. Krinsky

DAVID M. KRINSKY

ADDENDUM

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571-272-7822

Paper 41
Date: April 19, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

JSR CORPORATION and JSR LIFE SCIENCES, LLC,
Petitioner,

v.

CYTIVA BIOPROCESS R&D AB,
Patent Owner.

IPR2022-00036
IPR2022-00043
Patent 10,213,765 B2

Before ULRIKE W. JENKS, SHERIDAN K. SNEDDEN, and
SUSAN L. C. MITCHELL, *Administrative Patent Judges*.

JENKS, *Administrative Patent Judge*.

JUDGMENT
Consolidated Final Written Decision
Determining All Challenged Claims Unpatentable
35 U.S.C. § 318

IPR2022-00036
IPR2022-00043
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I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review of claims 1–7, 10–20, and 23–26 (“the challenged claims”) of U.S. Patent No. 10,213,765 B2 (Ex. 1001, “the ’765 patent”). We have jurisdiction under 35 U.S.C. § 6, and enter this Consolidated Final Written Decision pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons set forth below, we determine that JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) has shown, by a preponderance of the evidence, that the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e).

A. *Consolidated Proceedings*

The two captioned proceedings (IPR2022-00036 and IPR2022-00043 (or “the ’043 IPR”) involve the ’765 patent and challenge the same set of claims. The asserted grounds and prior art contentions are different in each proceeding. Consolidation is appropriate where, as here, the Board can more efficiently handle the common issues and evidence, and also remain consistent across proceedings. Under 35 U.S.C. § 315(d), the Director may determine the manner in which these pending proceedings may proceed, including “providing for stay, transfer, consolidation, or termination of any such matter or proceeding.” *See also* 37 C.F.R. § 42.4(a) (“The Board institutes the trial on behalf of the Director.”). There is no specific Board Rule that governs consolidation of cases. But 37 C.F.R. § 42.5(a) allows the Board to determine a proper course of conduct in a proceeding for any situation not specifically covered by the rules and to enter non-final orders to administer the proceeding. Therefore, on behalf of the Director under § 315(d), and for a more efficient administration of these proceedings, we

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consolidate IPR2022-00036 and IPR2022-00043 for purposes of rendering this Final Written Decision.

B. *Procedural History*

JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) filed a Petition for an *inter partes* review of claims the challenged claims under 35 U.S.C. § 311 in each proceeding. Paper 1¹ (“Pet.”). Petitioner supported the Petition with the Declaration of Dr. Steven M. Cramer. Ex. 1002. Cytiva Bioprocess R&D AB (“Patent Owner”) filed a Patent Owner Preliminary Response to the Petition. Paper 9 (“Prelim. Resp.”).

On April 21, 2022, pursuant to 35 U.S.C. § 314(a), we instituted trial (“Decision” or “Dec.” (Paper 10)) to determine whether any challenged claim of the ’765 patent is unpatentable.

In IPR2022-00036, Petitioner asserts the following grounds of unpatentability (Pet. 4):

Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–4, 12, 14–17, 25	103(a)	Linhult, Abrahmsén
1–7, 10–20, 23–26	103(a)	Linhult, Hober

¹ We note that the evidence filed in both proceedings is generally consistent in having the same exhibit number. Therefore, we reference exhibits and paper numbers as they appear in the record of IPR2022-00036, unless otherwise noted.

² The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the ’765 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–7, 10–20, 23–26	103(a)	Linhult, Abrahmsén, Hober
1–7, 10–20, 23–26	103(a)	Abrahmsén, Hober

In IPR2022-00043, Petitioner asserts the following grounds of unpatentability ('043 IPR Pet. 4):

Claim(s) Challenged	35 U.S.C. §³	Reference(s)/Basis
1-7, 10–20, 23–26	103(a)	Berg, Linhult
2, 3, 15, 16	103(a)	Berg, Linhult, Hober
1, 2, 5–7, 10–15, 18–20, 23–26	103(a)	Berg, Abrahmsén
2–4, 15–17	103(a)	Berg, Abrahmsén, Hober

Patent Owner filed a Patent Owner Response to the Petition. Paper 16 (“PO Resp.”). Patent Owner supported the Response with the Declaration of Dr. Daniel Bracewell (Ex. 2025). *See* PO Resp., iv (Exhibit List). Petitioner filed a Reply to the Patent Owner Response. Paper 29 (“Reply”). Petitioner supported the Reply with a Reply Declaration from Dr. Steven M. Cramer. Ex. 1061. Patent Owner filed a Sur-reply to Petitioner’s Reply. Paper 35 (“Sur-reply”).

³ The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the ’765 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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On February 16, 2023, the parties presented arguments at an oral hearing. Paper 36. The hearing transcript has been entered in the record. Paper 40 (“Tr.”).

For the reasons set forth below, we determine that Petitioner has shown by a preponderance of the evidence that claims 1–7, 10–20, and 23–26 of the ’765 patent are unpatentable.

C. *Real Parties in Interest*

Petitioner identifies itself, JSR Corporation and JSR Life Sciences, LLC, along with JSR Micro NV, as the real parties-in-interest. Pet. 2. Patent Owner identifies itself, Cytiva Bioprocess R&D AB, along with Cytiva Sweden AB and Danaher Corporation as real parties-in-interest. Paper 7, 1.

D. *Related Matters*

The ’765 patent is at issue in *Cytiva BioProcess R&D et al. v. JSR Corp. et al.*, Civil Action No. 1:21-cv-00310 (D. Del.). Pet. 2; Paper 5, 1.

In addition to the ’765 patent challenged here, Petitioner has filed Petitions for *inter partes* review of related U.S. patents as follows: U.S. Patent No. 10,343,142 B2 (“the ’142 patent”) in IPR2022-00041 and IPR2022-00044; and U.S. Patent No. 10,875,007 B2 (“the ’007 patent”) in IPR2022-00042 and IPR2022 00045. Pet. 2–3; Paper 7, 1–2. Petitioner indicates that the ’142 patent and the ’007 patent are also being asserted in the above-cited district court case. Pet. 3. The parties further list a pending application in the same family, U.S. App. Serial No. 17/107,600. Pet. 2; Paper 7, 2.

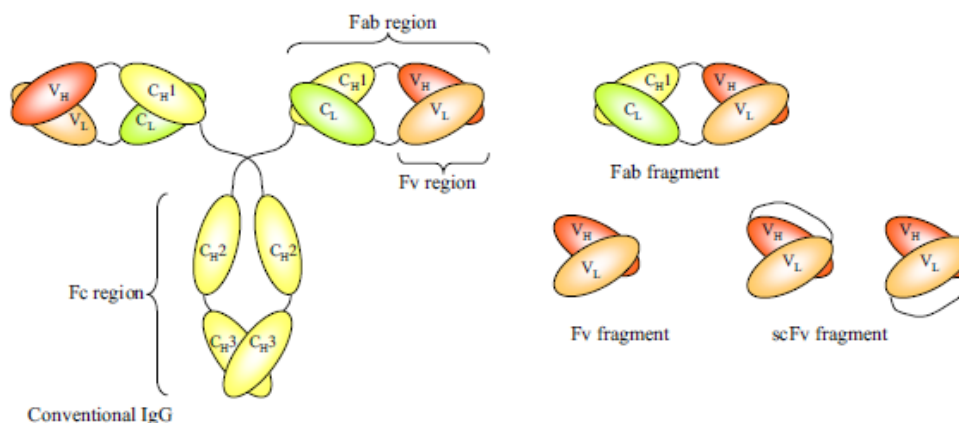
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E. *Subject matter background*

Antibodies (also called immunoglobulins) are glycoproteins, which specifically recognize foreign molecules. These recognized foreign molecules are called antigens. Ex. 2001, 1. A schematic representation of the structure of a conventional IgG and fragments is shown below:



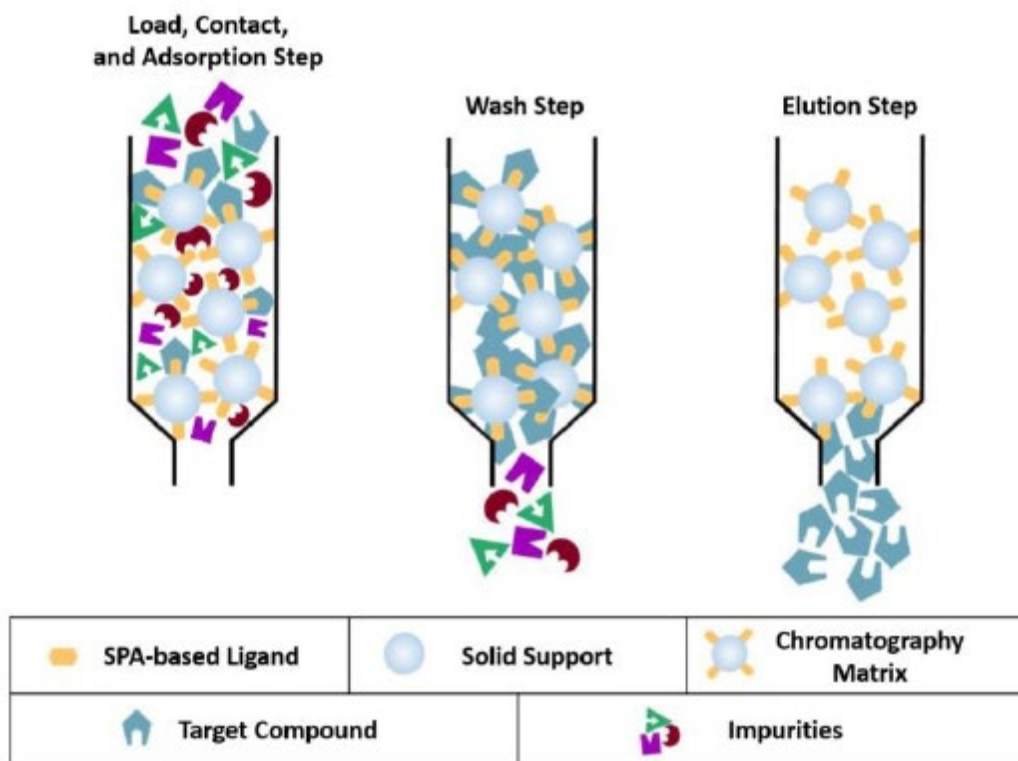
The figure (Ex. 2001, 2 (Fig. 1)), reproduced above, shows

the structure of a conventional IgG and fragments that can be generated thereof. The constant heavy-chain domains CH1, CH2 and CH3 are shown in yellow, the constant light-chain domain (CL) in green and the variable heavy-chain (VH) or light-chain (VL) domains in red and orange, respectively. The antigen binding domains of a conventional antibody are Fabs and Fv fragments. Fab fragments can be generated by papain digestion. Fvs are the smallest fragments with an intact antigen-binding domain. They can be generated by enzymatic approaches or expression of the relevant gene fragments (the recombinant version). In the recombinant single-chain Fv fragment, the variable domains are joined by a peptide linker. Both possible configurations of the variable domains are shown, i.e. the carboxyl terminus of VH fused to the N-terminus of VL and vice versa.

Ex. 2001, 2; *see also* PO Resp. 5.

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Below is a generic, exemplary schematic that shows how affinity purification typically works:



The figure shows the schematic of the loading, contact, and adsorbing step onto a column, followed by the wash step, and finally the elution and collection of the target compound. Ex. 1002 ¶ 24 (citing Ex. 1014 at §§ 1.1, 4.2.); *see also* PO Resp. 7 (“In a typical process, the composition containing the desired antibody then is loaded onto (i.e., pumped or injected into) the column.”); Pet. 6; *see generally* Ex. 1014.

F. *The '765 patent (Ex. 1001)*

The '765 patent is titled “Chromatography Ligand Comprising Domain C from *Staphylococcus Aureus* Protein A for Antibody Isolation.” Ex. 1001, (54). The '765 patent relates to an affinity ligand that is used for antibody isolation. *Id.* at 1:39–41. The '765 patent explains that

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chromatography is used in large-scale economic production of drugs and diagnostics in which proteins are produced by cell culture and then separated from the mixture of compounds and other cellular components to a sufficient purity. *Id.* at 1:52–61. One type of chromatography matrix for this purifying process includes immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG). *Id.* at 2:4–13. The '765 patent further explains that “[a]s in all process technology, an important aim is to keep the production costs low” by reusing matrices via cleaning protocols such as an alkaline protocol known as cleaning in place (CIP). *Id.* at 2:14–29. However, harsh treatments may impair the chromatography matrix materials such that there is a need for stability towards alkaline conditions for an engineered protein ligand. *Id.* at 2:31–48.

The '765 patent discloses that Protein A, known as SPA, is a constituent of the cell wall of the bacterium *Staphylococcus aureus*, and is widely used as a ligand in affinity chromatography matrices due to its ability to bind with IgG. *Id.* at 2:49–54. SPA is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind to antibodies at the Fc region, and it has been shown that each of these domains binds to certain antibodies at the Fab region. *Id.* at 2:54–63.

Domain C from SPA is defined by SEQ ID NO: 1 and is reproduced below.

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```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1           5           10           15
Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
                20           25           30
Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
            35           40           45
Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50           55

```

Id. at 4:27; 15:1–20. SEQ ID NO: 1 shows Domain C has Glycine (Gly) as an amino acid at the position 29 as annotated via red highlighting. According to the '765 patent, it has already been shown “that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A” and the '765 patent discloses that from experiments, “the present inventors have quite surprisingly shown that the SPA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product.”

Id. at 5:38–40, 51–55. The '765 patent discloses, “it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences -asparagine-glycine- and -asparagine-serine.” *Id.* at 5:62–66. The '765 patent then discloses “[q]uite surprisingly, the Domain C ligand of the invention presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29” and “[t]hus, in a specific embodiment, the chromatography ligand according to the invention comprises SPA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A.” *Id.* at 5:67–6:3, 6:49–52. The '765 patent discloses that a multimeric chromatography ligand (also denoted a “multimer”) can be comprised of at least two Domain C units and that a

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chromatography matrix can be comprised of ligands coupled to an insoluble carrier. *Id.* at 7:27–29, 8:21–23. The '765 patent discloses a column study of alkaline stability of its Protein A-derived ligands and a testing of the Fab binding of its ligands. *Id.* at 10:32–14:59 (Example 1). The study includes using an injection liquid and solution along with human normal immunoglobulin as a target compound in chromatography experiments, ligand coupling and column packing, adsorbance measurements, washing out unbound samples, and eluting bound material. *Id.* at 11:11–18, 12:25–34, 13:32–37.

1. *Illustrative Claim*

Claims 1 and 14 are the independent claims challenged by Petitioner in this proceeding. Independent claim 1, reproduced below, is illustrative of the subject matter:

1. A chromatography matrix comprising:

a solid support; and a ligand coupled to the solid support, the ligand comprising at least two polypeptides,

wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and

wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.

Ex. 1001, 15:39–48. Claim 14 is similar to claim 1 but recites “at least 55 amino acids in alignment with SEQ ID NO. 1” rather than “at least 55 contiguous amino acids of a modified SEQ ID NO. 1,” and wherein “each polypeptide has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1” rather than “the modified

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SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.” *Id.* at 16:51–60.

II. ANALYSIS

A. *Principles of Law*

“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify “with particularity . . . the evidence that supports the grounds for the challenge to each claim”)). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner must demonstrate by a preponderance of the evidence⁴ that the claims are unpatentable. 35 U.S.C. § 316(e); 37 C.F.R. § 42.1(d). A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time of the invention to a person having ordinary skill in the art. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations, including “the scope and content of the prior art”; “differences between the prior art and the claims at issue”; “the

⁴ The burden of showing something by a preponderance of the evidence requires the trier of fact to believe that the existence of a fact is more probable than its nonexistence before the trier of fact may find in favor of the party who carries the burden. *Concrete Pipe & Prods. of Cal., Inc. v. Constr. Laborers Pension Tr. for S. Cal.*, 508 U.S. 602, 622 (1993).

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level of ordinary skill in the art;” and “objective evidence of non-obviousness.” *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. *Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the “type of problems encountered in the art, [the] prior art solutions to those problems, [the] rapidity with which innovations are made, [the] sophistication of the technology, and [the] educational level of active workers in the field.” *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

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Petitioner asserts that a person of ordinary skill in the art would have had

(1) at least an advanced degree (*e.g.*, a Master’s or Ph.D.) in biochemistry, process chemistry, protein chemistry, chemical engineering, molecular and structural biology, biochemical engineering, or similar disciplines; (2) several years of post-graduate training or related experience (including industry experience) in one or more of these areas; and (3) an understanding of the various factors involved in purifying proteins using chromatography.[] Such a person would have had multiple years of experience with affinity ligand design and protein purification.

Pet. 10–11 (citing Ex. 1002 ¶¶ 13–14). Patent Owner does not dispute Petitioner’s definition of the person of ordinary skill. *See generally* PO Resp. Because Petitioner’s proposed definition is unopposed and appears consistent with the Specification and art of record, we apply it here.

C. *Claim Construction*

The Board applies the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.200(b) (2021). Under that standard, claim terms “are generally given their ordinary and customary meaning” as understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc).

Petitioner argues that based on Patent Owner’s implicit construction in the district court litigation “the term ‘the ligand comprising at least two polypeptides’ refers to a multimeric ligand (such as a tetramer) comprised of multiple polypeptides, each of which is a monomer.” Pet. 17 (citing Ex. 1020 ¶¶ 41, 50, 58, 62, 74, 87).

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Patent Owner does not contest Petitioner’s construction. *See generally* PO Resp.

According to the Specification, “a multimeric chromatography ligand (also denoted a ‘multimer’) comprised of at least two Domain C units, or a functional fragments or variants thereof.” Ex. 1001, 7:27–30. The Specification additionally recites that a multimer containing only Domain C units can, however, include linkers. *Id.* at 7:39–41. In addition, the Specification describes that “the multimer comprises one or more additional units, which are different from Domain C.” *Id.* at 7:44–45. Based on these disclosures in the Specification, a multimer is composed of at least two or more monomers.

Because Petitioner’s construction is consistent with the ’765 patent’s express construction of the term, and because Patent Owner does not contest Petitioner’s construction, we apply it here.

D. *Overview of Asserted References*

1. *Linhult (Ex. 1004)*

Linhult is titled “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach.” Ex. 1004, 1. Linhult discloses that due to the high affinity and selectivity of Staphylococcal protein A (SPA), “it has a widespread use as an affinity ligand for capture and purification of antibodies” but that “it is desirable to further improve the stability in order to enable an SPA-based affinity medium to withstand even longer exposure to the harsh conditions associated with cleaning-in-place (CIP) procedures.” *Id.*, Abst. Linhult discloses, “[t]o further increase the alkaline tolerance of SPA, we chose to

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work with Z, which is a small protein derived from the B domain of SPA.”
Id. at 2.

Figures 1A and 1B of Linhult are reproduced below.

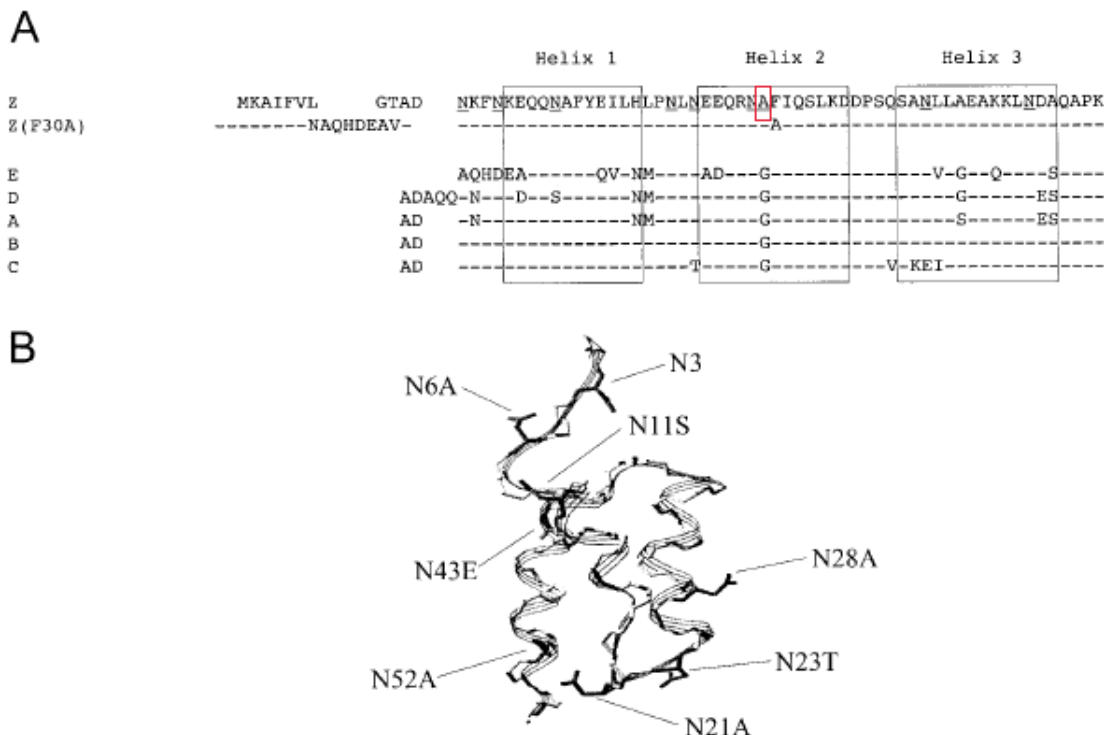


Figure 1A shows “[a]mino acid alignments of the Z, Z(F30A) and the five [naturally occurring] homologous domains (E, D, A, B, and C)” in which the horizontal lines indicate amino acid identity and “one glycine in the B domain [is] replaced [and] underlined” as annotated by the Board via a red box. *Id.* “Z(F30A), and all mutants thereof includes the same N-terminal as Z(F30A)” and “Z(N23T) was constructed with the same N-terminal as Z.” *Id.*⁵ Figure 1B shows “[t]he three-dimensional structure of the Z domain”

⁵ The mutation N23T having a change in amino acid correlates with the amino acid N next to the “Helix 2” box of Figure 1A as annotated by Petitioner. *See* Pet. 13.

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and “the different substitutions are indicated.” *Id.* Specifically, Linhult discloses,

[t]he B domain has been mutated in order to achieve a purification domain resistant to cleavage by hydroxylamine. An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine.[] Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.[] Protein Z is well characterized and extensively used as both ligand and fusion partner in a variety of affinity chromatography systems.

Id. Using a 0.5 M NaOH cleaning agent and “a total exposure time of 7.5 h for Z(F30A) and mutants thereof,” Linhult determines that “N23 seems to be very important for the functional stability after alkaline treatment of Z(F30A)” and “Z(F30A,N23T) shows only a 28% decrease in capacity despite the destabilizing F30A-mutation.” *Id.* at 4–5; Figs. 2, 3. Linhult reports that “[h]ence, the Z(F30A,N23T) is almost as tolerant as Z and is thereby the most improved variant with Z(F30A) as scaffold.” *Id.* at 5; Figs. 2, 3.

Linhult further discloses that “Z, Z(F30A), and mutated variants were covalently coupled to HiTrap™ affinity columns,” that “[t]he Z domain includes 8 asparagines (N3, N6, N11, N21, N23, N28, N43, and N52; Fig. 1),” and that “since the amino acid is located outside the structured part of the domain, it will most likely be easily replaceable during a multimerization of the domain to achieve a protein A–like molecule.” *Id.* at 4. Linhult confirms that “the affinity between Z(F30A) and IgG was retained despite the mutation.” *Id.* In Linhult’s studies, “[h]uman polyclonal IgG in TST was prepared and injected onto the columns in excess” and “[a] standard affinity chromatography protocol was followed.” *Id.*

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2. *Abrahmsén (Ex. 1005)*

Abrahmsén “relates to a recombinant DNA fragment coding for an immunoglobulin G ([I]gG) binding domain related to staphylococcal protein A . . . and to a process for cleavage of a fused protein expressed by using such fragment or sequence.” Ex. 1005, 1:8–13. Abrahmsén discloses that “[b]y making a gene fusion to staphylococcal protein A any gene product can be purified as a fusion protein to protein A and can thus be purified in a single step using IgG affinity chromatography.” *Id.* at 1:22–26. Abrahmsén explains that Protein A has “5 Asn-Gly in the IgG binding region of protein A” which “makes the second passage through the column irrelevant as the protein A pieces released from the cleavage will not bind to the IgG.” *Id.* at 1:58–63. Abrahmsén provides a solution to this problem “by adapting an IgG binding domain so that no Met and optionally no Asn-Gly is present in the sequence.” *Id.* at 1:64–67.

Abrahmsén discloses that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “[t]he number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén discloses that the recombinant DNA fragment can “cod[e] for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.” *Id.* at 2:32–37. According to Abrahmsén, from a computer simulation of the Gly to Ala amino acid change, it was “concluded that this change would not interfere with folding to protein A or binding to IgG.” *Id.* at 5:13–16.

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3. *Hober (Ex. 1006)*

Hober “relates to . . . a mutant protein that exhibits improved stability compared to the parental molecule” and “also relates to an affinity separation matrix, wherein a mutant protein according to the invention is used as an affinity ligand.” Ex. 1006, 1. Hober discloses that removal of contaminants from the separation matrix involves “a procedure known as cleaning-in-place (CIP)” but “[f]or many affinity chromatography matrices containing proteinaceous affinity ligands,” the alkaline environment “is a very harsh condition and consequently results in decreased capacities owing to instability of the ligand.” *Id.* at 1–2. According to Hober, structural modifications, such as deamidation and cleavage of the peptide backbone, of asparagine and glutamine residues in alkaline conditions is the main reason for loss of activity in alkaline solutions and that “the shortest deamidation half time have been associated with the sequences -asparagine-glycine and -asparagine-serine.” *Id.* at 2. Further, from a study of a mutant of ABD that was created, it was concluded that “all four asparagine residues can be replaced without any significant effect on structure and function.” *Id.* at 2–3. Hober points out that the SPA contains domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species and reagents of this protein with their high affinity and selectivity have found a widespread use in the field of biotechnology. *Id.* at 3. Accordingly, “there is a need in this field to obtain protein ligands capable of binding immunoglobulins, especially via the Fc-fragments thereof, which are also tolerant to one or more cleaning procedures using alkaline agents.” *Id.* at 4.

In one embodiment of Hober, a multimer “comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A” in which

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“asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids” for advantageous structural stability reasons wherein “the glycine residue in position 29 of SEQ ID NO: 1 has also been mutated, preferably to, an alanine residue.” *Id.* at 12. Hober’s SEQ ID NO: 1 is reproduced below.

```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1          5          10          15

Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln
          20          25          30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
          35          40          45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50          55

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Id. at SEQUENCE LISTING 1. SEQ ID NO: 1 shows a domain of *Staphylococcus aureus* having Glycine (Gly) as an amino acid at the position 29, as annotated via red highlighting.

Hober further discloses that its matrix for affinity separation “comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which protein at least one asparagine residue has been mutated to an amino acid other than glutamine.” *Id.* at 13. For its method of isolating an immunoglobulin, Hober discloses “in a first step, a solution comprising the target compounds, . . . is passed over a separation matrix under conditions allowing adsorption of the target compound to ligands present on said matrix” and “[i]n a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption, i.e. release of the target compound.” *Id.* at 16.

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E. *Obviousness in view of Linhult, Abrahmsén, and Hober*

1. *Petitioner's Contentions*

a) *Claims 1 and 14⁶*

Petitioner argues that “*Linhult* explains that SPA-based chromatography matrices have ‘widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.’” Pet. 19 (citing Ex. 1004, 1). Petitioner argues that *Linhult* teaches using a HiTrap™ chromatography affinity column made up of agarose beads that serve as a solid support for coupling SPA-based ligands. *Id.* (citing Ex. 1004, 4; Ex. 1002 ¶ 86). “*Linhult* discloses that its SPA-based ligands were ‘coupled to’ the solid support agarose beads [] contained in HiTrap™ affinity columns.” *Id.* at 20 (citing Ex. 1004, 4). Petitioner argues that “*Linhult* discloses that ‘multimerization’ of SPA monomers is performed to ‘achieve’ an “[SPA-]like’ affinity ligand.” *Id.* (citing Ex. 1004, 4; Ex. 1002 ¶¶ 91–93). Petitioner argues that “Figure 1(a), *Linhult* describes at least 55 amino acids of SPA’s naturally-occurring C domain (i.e., SEQ ID NO. 1).” *Id.* (citing Ex. 1004, 1, Fig. 1(a); see Ex. 1005, Fig. 2; Ex. 1006, Fig. 1; Ex. 1008, 639, Fig. 1). Petitioner argues “that all ‘five SPA domains show individual affinity for the Fc-fragment . . . as well as certain Fab-fragments of [antibodies] from most mammalian species.” *Id.* at 21 (citing Ex. 1004, 1). Petitioner argues that “*Abrahmsén* teaches a C(G29A)-based SPA ligand.” *Id.* (citing Ex. 1002 ¶¶ 99–111).

⁶ Petitioner treats claims 1 and 14 and their corresponding depend claims similarly; therefore, our analysis in this Decision groups the claims together.

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Petitioner argues that it was “known that individual SPA domains, including the C domain, could be used to construct SPA-based affinity ligands for purifying proteins.” *Id.* at 21 (citing Ex. 1002 ¶¶ 29, 101; Ex. 1004, 1; Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). Petitioner argues that “Linhult thus teaches a [person of ordinary skill in the art] that avoiding the Asn₂₈-Gly₂₉ dipeptide sequence through a G29A mutation, including on the C domain, would yield an SPA-based ligand having increased alkali-stability.” *Id.* at 22 (citing 1002 ¶¶ 100–04; Ex. 1011; Ex. 1012; Ex. 1013). Petitioner acknowledges that Linhult “does not expressly disclose a C(G29A)-based SPA ligand. Regardless, it would have been obvious to a [person of ordinary skill in the art] to modify *Linhult* based on the teachings of *Abrahmsén* to incorporate a C(G29A)-based SPA ligand in a chromatography matrix.” *Id.* at 23 (Ex. 1002 ¶¶ 99–111). Petitioner argues “*Abrahmsén* expressly discloses ‘a recombinant DNA coding for any of the E D A B C domains of [SPA], wherein the glycine codon(s) in the Asn_[28]-Gly_[29] coding constellation has been replaced by an alanine codon.’” *Id.* at 23 (citing Ex. 1005, 2:32–37).

Petitioner concludes that

applying the teachings of *Abrahmsén* with *Linhult* would have involved merely combining known elements in the field (e.g., an affinity chromatography matrix comprising a G29A-containing ligand coupled to a solid support, as in *Linhult*, and a C(G29A)-based amino acid sequence, as in *Abrahmsén*) according to known ligand-construction methods to yield a predictable results (e.g., the claimed affinity chromatography matrix).

Pet. 24.

Petitioner argues that *Abrahmsén* “unequivocally discloses including a G29A mutation on SPA’s C domain.” Pet. 49 (citing Ex. 1005, 2:32–37,

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5:13–16; Ex. 1002 ¶¶ 222–27). The Petition recognizes that in Abrahmsén an IgG chromatography column was used to purify the recombinantly produced SPA. *Id.* In other words, the column matrix had IgG bound to the matrix and could capture the recombinantly produced SPA binding domains.

Petitioner relies on Hober for teaching that the substitution in G29A of SEQ ID NO: 1 would have been obvious and to address the conventional features set forth in dependent claims. *See* Pet. 20–26, 48–49. Specifically, Petitioner argues that Hober teaches “that naturally-occurring SPA ‘contain[s] domains capable of binding to the Fc and Fab portions’ of antibodies.” Pet. 33 (citing Ex. 1006, 3); Ex. 1006, 3 (“An example of such a protein [used for affinity chromatography] is staphylococcal protein A, containing domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species.”). Petitioner argues that Hober also teaches that the shortest deamidation half lives are seen with sequences that contain an Asn-Gly dipeptide. Pet. 33 (citing Ex. 1005, 2); Ex. 1002 ¶ 76 (“*Hober* teaches that Asn-Gly dipeptide sequences are associated with ‘the shortest deamidation half times,’ which is a degradation process that occurs at high pH conditions.”), *see id.* ¶ 141 (deamidation “is a process that degrades SPA-based ligands at high pH conditions.”).

According to Petitioner, “*Hober* discloses, for example, that ‘SPA-based affinity medium probably is the most widely-used affinity medium for isolation of monoclonal antibodies and their fragments from different samples.’” Pet. 50 (citing Ex. 1006, 3).

Hober states, for example, that SPA-based monomers “can be combined into multimeric proteins, such as dimers, trimers, tetramers, pentamers etc.” (Ex. 1006, 11; *see also* Ex. 1005, 9:14–10:41 (*Abrahmsén* disclosing Example V on “Construction

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of Dimeric Z Fragment”).) A [person of ordinary skill in the art] would have a good reason to combine the teaching from *Abrahmsén* with the teachings of *Hober* since multimeric SPA-based ligands allowed for additional surface area to bind target compounds. (Ex. 1002 ¶ 239.)

Pet. 52–53. Petitioner’s declarant, Dr. Cramer explains that using the ligand as a multimeric ligand has “the benefits of additional surface area on the SPA-based ligand to bind target compounds.” Ex. 1002 ¶ 239 (citing Ex. 1032, 560). Abrahmsén discloses that SPA domain C as shown in Figure 2 (*see* Ex. 1005 at col. 3:25-35, Fig. 2) has at least 55 contiguous amino acids of SEQ ID NO. 1. *See* Ex. 1002 ¶¶ 242–244. “*Abrahmsén* further confirms there are no structural integrity concerns in constructing a C(G29A)-based SPA ligand by disclosing that incorporation of a G29A mutation ‘would not interfere with folding [of SPA] or binding to [antibodies].’” Ex. 1002 ¶ 246.

b) Claims 4 and 17

With respect to claims 4 and 17, Petitioner argues that the property of being “capable” of binding to the Fab fragment of an antibody is an inherent property of SEQ ID NO:1 that has the glycine exchanged for the alanine at position 29. *See* Pet. 28. Specifically, Petitioner argues that “*Linhult* discloses that it was well known by 2004 that the five domains of SPA, including the C domain, ‘show individual *affinity for*...certain *Fab-fragments* of [antibodies] from most mammalian species.’” Pet. 29 (citing Ex. 1004, 1; Ex. 1002 ¶ 125). “Even if the capability to bind the Fab part of an antibody is not an inherent property of the claimed C(G29A)-based SPA ligand, *Linhult* discloses this limitation.” *Id.* Petitioner notes further that “dependent claims 4 and 17 permit the incorporation of additional amino

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acid sequences known to possess the claimed property.” *Id.* (citing Ex. 1002 ¶ 126).

c) Claims 2, 3, 12, 15, 16, and 25

With respect to claims 2, 3, 12, 15, 16, and 25, Petitioner directs our attention to where in the record the various limitations of the dependent claims may be found. *See* Pet. 25–28, 30. Specifically, Petitioner asserts that Linhult describes multimerization of the ligand as recited in claims 2, 12, 15, and 25. Pet. 26 (citing Ex. 1004, 410; Ex. 1002 ¶¶ 112–114), *see id.* at 30 (citing Ex. 1002 ¶¶ 128–129). Because the wild-type C-domain-based multimeric ligand inherently possesses the claimed binding-capacity property as recited in claims 3 and 16, there is a reasonable expectation that a chromatography possessing the C(G29A)-mutation would achieve similar binding capacity. Pet. 27–28 (citing Ex. 1002 ¶¶ 115–119).

2. Patent Owner’s Contentions

Patent Owner argues that the Petition fails to demonstrate that it would have been obvious to make the chromatography matrix as claimed (PO Resp. 17–38); that the Petition has not established that there is a reasonable expectation of success in arriving at the claimed matrix (*id.* at 47–52); that the art teaches away from making the G29A modification (*id.* at 38–41); that the artisan would not have been motivated make additional mutations (*id.* at 43–44); and that objective indicia supports a conclusion of non-obviousness (*id.* at 53–53).

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a) Matrix

According to Patent Owner, “Petitioners fail to explain why the POSA would have been motivated to select Domain C’s amino acid sequence as the foundation for an engineered SPA ligand with favorable properties.”

PO Resp. 18. Specifically arguing that the obviousness analysis requires the prior art be viewed as a whole. PO Resp. 20 (citing *In re Wesslau*, 353 F.2d 238 (CCPA 1965); *In re Enhanced Sec. Rsch., LLC*, 739 F.3d 1347, 1355 (Fed. Cir. 2014); *Impax Lab ’ys Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018)).

Patent Owner argues that because nobody was working on Domain C at the time the invention was filed, therefore, the selection of Domain C for further development could not possibly be obvious. *See* PO Resp. 23 (“Reliance on *KSR* also is foreclosed by the evidence that no one in the art was seeking to modify Domain C.”), *see also id.* at 24 (“But no prior art cited by Petitioners singles Domain C out for further development. Ex. 2025 ¶¶ 89-96”), *id.* at 26 (“Dr. Cramer [Petitioner’s expert] himself highlights, it would have been natural for the POSA to further develop the domain—Domain B—that was best understood and for which there was a crystal structure available. Ex. 1002 ¶ 33; Ex. 2015 at 137:20–138:19; Ex. 2017”), *id.* at 28 (“The notion that this body of work would lead the POSA to discard the improved ligands the references themselves focus on, and instead start experimenting with mutations to Domain C—strains credulity. Ex. 2025 ¶¶ 92–95”).

According to Patent Owner, neither Linhult nor Abrahmsén supply the motivation to start with Domain C. “Linhult focuses exclusively on, and concerns improvements to, the alkaline stability of Domain Z by mutating

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asparagine residues. *See* Ex. 2025 ¶¶ 64-67, 92.” PO Resp. 29. “Rather than use Domain C, the POSA reviewing Linhult would be motivated to keep working with Domain Z, adopting the N23T mutation. Ex. 2025 ¶ 67 & n.3.” PO Resp. 30. “Neither Abrahmsén itself nor the Petition provide any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domains. *WBIP[LLC v. Kohler Co.]*, 829 F.3d 1317, 1337 (Fed. Cir. 2016)].” PO Resp. 31.

b) Reasonable Expectation of Success

Patent Owner argues that “the field of protein engineering is notoriously unpredictable.” PO Resp. 21 (citing Ex. 2025 ¶¶ 50-52). Arguing that “despite their supposed structural similarity, there are a number of differences between the naturally-occurring domains of protein A, including five different amino acids in the sequences of Domain B (with which the industry was quite familiar) and Domain C (which remained virtually ignored as of the priority date).” *Id.* at 22 (citing Ex. 2025 ¶ 48).

Protein engineering is a highly complex and unpredictable field and was all the more so as of the priority date more than fifteen years ago. *See, e.g.,* Ex. 2025 ¶¶ 50-52. . . . As amply demonstrated by the effect of the G29A mutation on Domain Z’s Fab-binding ability, even a single amino acid substitution can drastically alter the properties of a protein. Ex. 2025 ¶ 52; Ex. 2015 at 51:15-52:1 ([Dr. Cramer, Petitioner’s expert] agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability), 18:10-12, 73:16-20.

PO Resp. 34–35.

Patent Owner argues that

The Federal Circuit has rejected arguments premised on the notion that a homologous structure renders an invention obvious, particularly given the difficulty and uncertainty in the art as of

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the priority date. *See, e.g., Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208 (Fed. Cir. 1991) (holding the use of a monkey gene to probe for a roughly 90 percent “homologous” human gene would not have been obvious, particularly given expert testimony that isolating a particular gene would have been “difficult” and the lack of certainty in the endeavor).

PO Resp. 36. Specifically, Patent Owner argues that the Fab-binding capability of a ligand could not have been predicted and therefore there is no reasonable expectation of success. *See* PO Resp. 38.

c) Teaching Away

Patent Owner argues that the prior art would have told the person of ordinary skill in the art to avoid a G29A a modification to Domain C.

PO Resp. 38. In other words, its Patent Owner’s contention is that the prior art teaches away from making this modification. “The very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding ‘negligible’ when implemented in Domain B.” PO Resp. 40. Patent Owner argues that a person seeking to improve Fab binding one avoid a G29A substitution of Domain C. PO Resp. 40–41.

d) Additional Modifications

Patent Owner argues that “the prior art would have taught the POSA to make asparagine substitutions, not glycine substitutions, to address alkaline stability concerns.” PO Resp. 43. In other words, Patent Owner’s argues the prior art would have suggested making additional substitutions most notably in the asparagine residues of Domain C. *Id.* at 44.

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e) Inherency

Patent Owner argues that Petitioner cannot rely on inherency to circumvent that the combination lacks a reasonable expectation of success. PO Resp. 52 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. DE C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017)). “As Dr. Bracewell [Patent Owner’s expert] explains, the POSA did not know and could not have known the properties of a modified Domain C ligand, since the effects of even single-amino acid substitutions are undisputedly unpredictable and the cited art includes absolutely no protein engineering work with Domain C. Ex. 2025 ¶¶ 120-122.” PO Resp. 48. According to Patent Owner, because nobody was working on domain C the person of ordinary skill in the art “is simply left to guess at how such a ligand would perform.” PO Resp. 49 (citing Ex. 2025 ¶¶ 121–124). Specifically, the person of ordinary skill in the art would not know whether a Domain C-G29A mutation would be capable of binding Fab part of an antibody. *Id.*

f) Unexpected Results

Patent Owner argues that it was wholly unexpected that the modified C(G29A)-based SPA ligand to bind the Fab part. PO Resp. 55. “[T]he SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” PO Resp. 55.

3. Petitioner’s Reply

In response, Petitioner argues that

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Abrahmsén and *Hober* each expressly pointed to a C(G29A) mutation (Ex. 1005, 2:32-37; Ex. 1006, 12), which was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence (*see, e.g.*, Ex. 1004, 408). As the Board recognized, “*Abrahmsén* provides motivation for making [the G29A] mutation in **any of the IgG binding domains**.” (Decision, 24; *see also* Ex. 1057, 97:3-16 (Dr. Bracewell admitting that *Abrahmsén* discloses a G29A mutation to any of the five domains, including Domain C).)

Reply 2.

A POSA would have reasonably expected success in combining these teachings to achieve the claimed affinity chromatography matrix given the well-known fact that each individual domain, including Domain C, has affinity for antibodies (Ex. 1004, 407), as well as *Abrahmsén*’s confirmation that G29A “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1057, 99:13-101:21 Ex. 1005, 5:13-16; Ex. 1002 ¶110).

Id. at 3.

Petitioner argues that Patent Owner “has not disputed that *Abrahmsén* disclosed that G29A ‘would not interfere with folding to protein A or binding to IgG.’ (Ex. 1005, 2:32-37, 5:4-16; Ex. 1057, 109:20-110:17.) Nor does it take issue with its own statements in *Hober* that G29A is advantageous for ‘structural stability reasons.’ (Ex. 1006, 12.)” *Id.* at 9. “*Abrahmsén* and *Hober*, which make clear that G29A does not affect the ability of an SPA ligand to bind to an antibody. (Ex. 1005, 2:32-37, 5:4-16; Ex. 1006, 12.)” *Id.* at 10.

Petitioner argues that “a POSA would have started with any one of the naturally occurring domains. (Decision, 26-28.) To then increase alkali stability, a POSA would have made the simplest, well-known substitution: G29A. (Section II.A.1-2; Ex. 1061 ¶¶8-15.)” Reply 11.

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Petitioner argues “that Fab-binding was an inherent feature of a C(G29A)-based ligand—which [Patent Owner] does not appear to dispute. (Decision, 30; [PO] Resp., 52-53.) In fact, [Patent Owner] acknowledges that ‘C(G29A)-based SPA ligands retained substantial Fab-binding ability.’ (Resp., 55.)” *Id.* at 14.

Petitioner argues that “Fab-binding is not being used [in the Petition] as part of a finding of a motivation to combine; rather, it is an inherent property [of the composition] being claimed. And necessarily present properties do not add patentable weight when they are claimed as limitations. *In re Kubin*, 561 F.3d 1351, 1357 (Fed. Cir. 2009).” Reply 15–16. Petitioner further argues that Patent Owner’s reliance on *Honeywell* is misplaced because “*Honeywell* had to do with an inherent property being used as a teaching in an obviousness analysis; it did not involve a limitation in the challenged claim reciting an inherent property.” Reply 15 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. De C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017); *see also Pernix Ireland Pain v. Alvogen Malta Operations*, 323 F. Supp. 3d 566, 607(D. Del. 2018)).

4. Patent Owner’s Sur-reply

Patent Owner argues that “Petitioners, and the Institution Decision, overlook an important point of consensus between the parties’ experts: the field of protein engineering is notoriously *unpredictable*.” Sur-reply 2. Patent Owner maintains that Petitioner has not identified a motivation to start from Domain C. *Id.* at 3. Patent Owner argues that “Petitioners would have the Board look past the multitude of references teaching a preference for Domains B and Z—including Petitioners’ foundational references—and

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seize upon fleeting mentions of Domain C.” *Id.* at 7 (citing *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965)). Patent Owner argues that “[m]ere sequence homology does not make the field predictable, as both experts observe, Ex. 2015 at 51:15-52:1, 56:4-12, 75:12-22, 73:16-20; Ex. 2025 ¶¶ 50-52; Ex. 2049 at 72:1-73:12, and as the vastly different Fab-binding properties of the near-identical Domains B and Z well illustrate, Ex. 2029 at 8.” *Id.* at 8–9.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

5. Analysis

a) Claims 1 and 14

Claims 1 and 14 of the ’765 patent are directed to a composition. Specifically, a chromatography matrix (i.e. a solid support) that has a ligand attached, and that ligand is made up of at least two polypeptides comprising 55 contiguous amino acids of SEQ ID NO: 1⁷ having a G29A mutation. *See* Ex. 1001, 15:39–48.

Linhult teaches that SPA is a cell surface protein expressed by *Staphylococcus aureus* and consists of five highly homologous domains (EDABC). Ex. 1004, 1. Each of “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as

⁷ Wild type amino acid sequence of domain C from *Staphylococcus* protein A (SPA). *See* Ex. 1001, 4:27, 6:35–36, 6:51–52.

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well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” *Id.* “Due to the high affinity and selectivity of SPA, it has a widespread use as an affinity ligand for capture and purification of antibodies.” Ex. 1004, Abst., *see also id.* at 1 (“SPA has a widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.”).

Linhult explains that, in column chromatography, sodium hydroxide (NaOH) is probably the most extensively used cleaning agent for removing contaminants such as nucleic acids, lipids, proteins, and microbes, and a cleaning-in-place (CIP) step is often integrated in the protein purification protocols using chromatography columns. Ex. 1004, 1. “Unfortunately, protein-based affinity media show high fragility in this extremely harsh environment, making them less attractive in industrial-scale protein purification. SPA, however, is considered relatively stable in alkaline conditions.” *Id.* at 2. Linhult teaches that the combination of “[a]sparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.” *Id.* Linhult teaches that “[a]n exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is [also] a cleavage site for hydroxylamine.” *Id.*

Petitioner’s declarant, Dr. Cramer explains that the “Z” domain referenced in Linhult refers to a synthetic version of the wild-type (i.e., natural) B domain of SPA, in which the naturally occurring glycine in the Asn₂₈-Gly₂₉ dipeptide sequence is replaced by an alanine residue to create an Asn₂₈-Ala₂₉ dipeptide sequence. Ex. 1002 ¶ 30 (citing Ex. 1004, 408, Fig. 1(a); Ex. 1007, 109, Fig. 1); ¶ 31 (citing Ex. 1005).

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We credit Dr. Cramer for establishing that the C domain sequence disclosed in Linhult contains 55 amino acids of domain C as recited in SEQ ID NO: 1. Ex. 1002 ¶ 97 (showing a sequence alignment), *see also id.* ¶ 243 (showing sequence alignment of domain C of Abrahmsén with SEQ ID NO: 1); *see also* Ex. 2025 ¶ 41 (Patent Owner’s declarant Dr. Bracewell showing SPA sequence alignment for Domains A, B, C, D, E, and Z).

Linhult teaches making affinity chromatography columns with protein Z, Z(F30A), and additional mutated variants. These modified proteins were covalently attached to HiTrap™ columns in Linhult using NHS-chemistry. Ex. 1004, 4. Human polyclonal IgG was prepared and injected onto the columns in excess and “[a] standard affinity chromatography protocol was followed.” *Id.* at 4. Linhult exemplifies using the Z domain and Z domain mutants attached to the column for the isolation of IgG from a sample. *Id.* Linhult, therefore, teaches column chromatography matrix that has a SPA domain containing a G29A mutation attached to a chromatography matrix.

According to Abrahmsén, the IgG binding domains of E D A B C domains of SPA were known. *See* Ex. 1005, 3:25–35, 4:34–37, Fig. 2. Abrahmsén teaches that the Asn-Gly dipeptide is located in the middle of an alpha helix involved in the binding to IgG. *Id.* 4:56–60; Ex. 2025 ¶ 41 (showing alpha helix regions in SPA domains). Relying on “computer analysis [Abrahmsén] surprisingly showed that the Gly in the Asn-Gly dipeptide sequence could be changed to an Ala. This change was not obvious as glycines are among the most conserved amino acids between homologous protein sequences due to their special features.” Ex. 1005, 5:7–9. Abrahmsén teaches that “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23.

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Abrahmsén teaches “a recombinant DNA fragment coding for *any of the E D A B C domains* of Staphylococcal protein A, wherein the *glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.*” *Id.* at 2:33–37 (emphasis added). Abrahmsén, therefore, provides motivation for making this mutation in *any one* of the IgG binding domains of E D A B C domains of SPA.

Abrahmsén, like Linhult, only exemplifies the cloning and expression of the Z-domain, which is a B-domain with a G29A mutation. Ex. 1005, 7:65–10:56. A references disclosure, however, is not limited only to its preferred embodiments, but is available for all that it discloses and suggests to one of ordinary skill in the art. *In re Lamberti*, 545 F.2d 747, 750 (CCPA 1976); *see also In re Susi*, 440 F.2d 442, 446 n.3 (CCPA 1971) (disclosed examples and preferred embodiments do not constitute a teaching away from a broader disclosure or non-preferred embodiments). Here, Abrahmsén expressly suggests making the same mutation in *any one* of the SPA domains E D A B C. Ex. 1005, 2:33–37

Thus, the disclosures of both Linhult and Abrahmsén suggest mutating the glycine at position 29 for an alanine in an any one of the SPA IgG binding domains of E D A B or C in order to avoid protein degradation in alkaline conditions and degradation by hydroxylamine. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”); Ex. 1005, 4:56–57 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine.”); Ex. 1006, 2 (“and the shortest deamidation half times

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have been associated with the sequences –asparagine–glycine and –asparagine–serine”); Reply 3–5; Ex. 1061 ¶¶ 3–6.

Based on these disclosures, the combined teachings of Linhult and Abrahmsén suggest making the G29A mutation in *any one* of the SPA IgG binding domains E, D, A, B, or C. We, therefore, agree with Petitioner that attaching any other mutated SPA IgG binding domains E, D, A, or C using “known ligand-construction methods to yield a predictable result[] (e.g., the claimed affinity chromatography matrix)” would have been obvious. Pet. 24 (citing Ex. 1002 ¶ 109). As the Federal Circuit has explained, “[w]here a skilled artisan merely pursues ‘known options’ from ‘a finite number of identified, predictable solutions,’ the resulting invention is obvious under Section 103.” *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421).

Accordingly, we agree with Petitioner that the combination of Linhult and Abrahmsén expressly suggests mutating the glycine codon for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111).

We address Patent Owner’s contentions below.

(1) Matrix

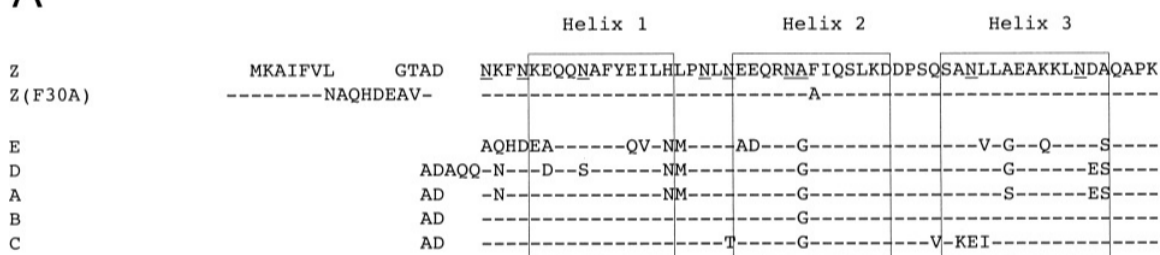
We do not find Patent Owner’s argument that the Petition fails to identify a reason to select domain C persuasive. PO Resp. 17–30. Specifically, we are not persuaded by Patent Owner’s contention that just because nobody was working on Domain C at the time the invention was filed, therefore, selection of Domain C cannot be obvious. *See* PO Resp. 31

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(“A general recognition that there exist five naturally occurring protein A domains is not a motivation to use each of them as a starting point for the claimed mutations”).

Petitioner’s articulated obviousness ground is premised on the knowledge that *any one* of the five SPA IgG binding domains are known to bind IgG and can function as a ligand for the purification of antibodies. Linhult and Abrahmsén both expressly suggest that the glycine codon at position 29 can be mutated for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Ex. 1004, 2; Ex. 1005, 2:32–37. The SPA IgG binding domains comprise a short list of 5 members: E, D, A, B, or C. Of these 5 members, the glycine at position 29 in Domain B has already been mutated to an alanine to create a Domain Z which has been shown to retain IgG binding activity. Ex. 1004, 6 (Fig. 3). Figure 1A of Linhult is reproduced below.

A



Linhult’s Figure 1A, reproduced above, shows the amino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C). The three boxes show the α -helices. Ex. 1004, 2; Ex. 1005, Fig. 2.

As discussed in our Institution Decision (Dec. 26–27), “it is fair to say that there were ‘a finite number of identified, predictable solutions’ to the problem of finding” a SPA IgG binding domain that is resistant to protein degradation by mutating the glycine at position 29 for an alanine and this is

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a “product not of innovation but of ordinary skill and common sense.” *See Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421). It is well established that

[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties.

In re Deuel, 51 F.3d 1552, 1558 (Fed. Cir. 1995). Here, Linhult and Abrahmsén show that the IgG binding domains of SPA – E, D, A, B, or C share many structural similarities. *See* Ex. 1004, 2 (Fig. 1(a) (reproduced above)); Ex. 1005, 3:25–35.

There is also an express teaching in both Linhult and Abrahmsén to mutate the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which supports the obviousness of incorporating the mutation into any IgG binding domain that has the Asn-Gly dipeptide. *See, e.g., SIBIA Neurosciences, Inc. v. Cadus Pharm. Corp.*, 225 F.3d 1349, 1358–59 (Fed. Cir. 2000) (stating that an express teaching in the prior art suggesting a particular modification establishes obviousness).

Accordingly, we find that Petitioner has shown by a preponderance of the evidence that the combined teachings of Linhult and Abrahmsén suggests the use of *any one* of the SPA IgG binding domains E, D, A, B, or C as the starting ligand for purifying IgG antibodies, and that making the G29A mutation in *any one* of the domains would have been obvious because it would provide ligands that are less susceptible to alkaline conditions and

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are resistant to hydroxylamine cleavage. Pet. 21–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

(2) Reasonable Expectation of Success

We are not persuaded by Patent Owner’s contention that there is no reasonable expectation of success in using a G29A mutation in Domain C. PO Resp. 39–40; 47–53.

Linhult explains that removing the asparagine–glycine amino acid combination not only results in the removal of the hydroxylamine cleavage site but also creates a product that is more alkaline resistant. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”).

Abrahmsén teaches that this Asn-Gly amino acid combination is present in all five IgG binding domains and that mutating the dipeptide would not interfere with IgG binding. Ex. 1005, 4:56–58 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine. As this sequence is kept intact in all five IgG binding domains of protein A. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”).

Abrahmsén’s conclusion that the mutation would not interfere with binding to IgG is supported by Linhult (*see* Ex. 1004, 6 (Fig. 3)) and Jansson.⁸

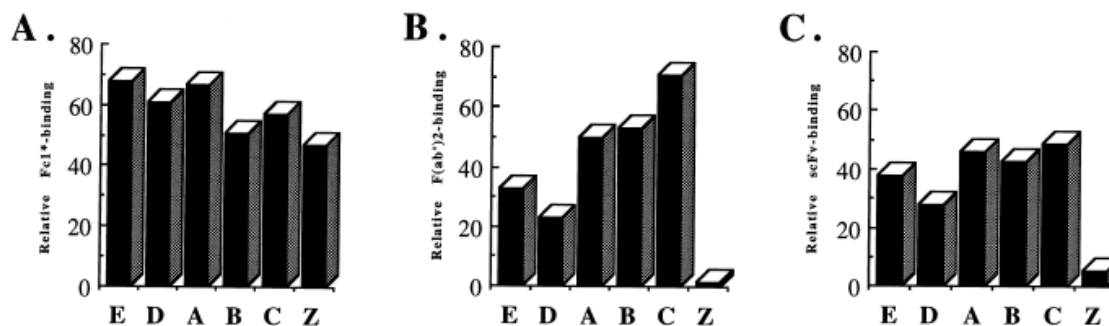
⁸ Patent Owner cites Jansson for the position that Domain Z has negligible binding to Fab. *See* PO Resp. 39–40. Claims 1 and 14 are not limited to Fab binding, indeed the claims do not even require IgG binding.

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Jansson's Fig. 3, reproduced below, shows a side-by-side comparison of Fc1*, Fab, and scFv binding.



Jansson Figure 3 (Panel A), reproduced above, shows that the single G29A mutation between Domain B and Domain Z results in a protein that is able to bind IgG.⁹ Comparing panel A–B column with panel A–Z column, the relative binding capacity in both columns remains close to 50% for both, indicating that the G29A mutation does not interfere with IgG binding. Ex. 2009, 6. This is a result already predicted by Abrahmsén's computer modeling and substantiated by Linholt. *See* Ex. 1005, 4:56–58; Ex. 1004, 6 (Fig. 3).

“Obviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success.” *In re Droge*, 695 F.3d 1334, 1338 (Fed. Cir. 2012) (quoting *In re Kubin*, 561 F.3d 1351, 1360 (Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894, 903–04

⁹ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the “IgG control” in Jansson. Patent Owner's counsel explains that “Part A is Fc binding. So that is, I believe the way they did this experiment was with Fc fragments, but it's generally acknowledged, you know, these antibodies all have an Fc domain if they're a whole antibody and that reflects the fact that all of these domains A, B, C, D and E and domain Z, which is B with the G29A mutation, retain this Fc binding.” Tr. 70:6–11.

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(Fed.Cir.1988)); *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016) (explaining that the expectation of success issue involves a showing of “a reasonable expectation of achieving *what is claimed*”) (emphasis added). It is not inventive to confirm something that was already suggested in the art. “Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention.” *Pharma Stem Therapeutic, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1363–1364 (2007).

Here, the record supports that each individual SPA domain, including the C domain, has affinity for IgG antibodies. Ex. 1004, 1 (“The five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” (bracketing in original)). Abrahmsén suggests making the mutation of Asn-Gly coding constellation in *any one* of the SPA domains by replacing glycine with an alanine codon that would remove the dipeptide sequence known to be sensitive to hydroxylamine degradation. *See* Ex. 1005, 4:56–5:16, *see also id.* Fig. 2 (showing the Asn-Gly coding constellation in all SPA domains); Ex. 1006, 2 (“the shortest deamidation half times have been associated with the sequences – asparagine–glycine and – asparagine–serine”). Abrahmsén’s confirms that a G29A mutation on SPA would not interfere with folding of SPA protein and the binding to antibodies. Ex. 1005, 5:13–16 (“by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”). Abrahmsén’s computer modeling suggests that IgG binding is not impacted by the mutation and this is confirmed by Linholt’s experiments showing that the

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G29A mutant of Domain B (a.k.a. Domain Z) binds IgG. Ex. 1004, 6 (Fig. 3).

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

We are not persuaded by Patent Owner’s contention that the information gained by computer modeling of the SPA native domain B – IgG crystal structure could not be extrapolated to other SPA domains that are structurally very similar.

As Petitioner’s expert, Dr. Cramer explains

It was well known that the researchers who developed the Z domain based on the wild-type B domain (rather than any of the other four SPA domains) did so for two reasons. (*See, e.g.*, Ex. 1007 at 109.) First, a crystal structure of the wild-type B domain binding to an antibody happened to be available in 1981 for analysis. (*See, e.g., id.*; Ex. 1005 at col. 4:56-68; Ex. 1017.) And, second, *their work would be informative of mutations that could be done on all five of the highly homologous SPA domains more generally.* (*See, e.g.*, Ex. 1005 at col. 2:32-37; Ex. 1007 at 109; Ex. 1008 at 639, Fig. 1.)

Ex. 1002 ¶ 33 (emphasis added). Dr. Cramer further explains that “[t]hey did the computer modeling based on that complex because that’s the crystal structure that they had. It wasn’t done because the B domain is special. . . . And then there’s several other places where they state clearly that they could also do the other domains with expected similar results.” Ex. 2015, 138:8–22.

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Accordingly, we agree with Petitioner that taken together, the teachings of Linhult, Abrahmsén, and Hober provide a reasonable expectation of success at arriving at a chromatography composition that contains the SPA domain C ligand with the G29A mutation. Pet. 25 (citing Ex. 1005, 5:13–16; Ex. 1002 ¶ 110).

(3) No Teaching Away

We are also not persuaded by Patent Owner’s contention that the art teaches away from the G29A substitution because it interferes with Fab binding. *See* PO Resp. 38–40; Sur-reply 9; Ex. 2009 at 2; Ex. 2010 at 25; Ex. 2012 at 25–26; Ex. 2029 at 7.

Neither claim 1 nor claim 14 recite a need to bind the Fab region of an antibody – all that is required by these claims is a chromatography matrix with a domain C ligand attached, and the domain C sequence having a G29A mutation.¹⁰ The law does not require that the teachings of the reference be combined for the reason or advantage contemplated by the inventor, as long as some suggestion to combine the elements is provided by the prior art as a whole. *In re Beattie*, 974 F.2d 1309, 1312 (Fed. Cir. 1992); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976); *see In re Kemps*, 97 F.3d 1427, 1430 (Fed. Cir. 1996) (“[T]he motivation in the prior art to combine the references does not have to be identical to that of the applicant to establish obviousness.”).

Here, Linhult teaches that “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most

¹⁰ We note that neither claim 1 nor claim 14 even requires IgG binding.

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mammalian species.” Ex. 1004, 1 (bracketing in original) (citation omitted). Linhult, therefore, teaches that *any one* of the SPA IgG binding domains E, D, A, B, or C can bind the Fc region of an antibody and can thereby be used as a ligand for purifying IgG antibodies. In addition, the combination of Linhult and Abrahmsén suggests making the G29A mutation in each of the domains because it would provide ligands that are less susceptible to protein degradation. Ex. 1004, 2; *see also* Ex. 1005, 2:33-37 (“[A] recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.”).

Patent Owner contends that the G29A mutation would lead to a reduction in the Fab binding of domain C, and therefore, would lead away from making the mutation. PO Resp. 40–41 (citing Ex. 2010 at 2; Ex. 2011 at 25; Ex. 2012 at 25–26; Ex. 2013 at 2–3; Ex. 2025 ¶¶ 105–109; Ex. 2029 at 6–7). Patent Owner’s cited references are directed to Fab binding. But claims 1 and 14 are not limited to Fab binding. Showing that the G29A mutation interferes with Fab binding says nothing about the ability of a mutated SPA domains E, D, A, B, or C to bind the Fc portion of IgG. *See, e.g.*, Ex. 2013, 3 (“The site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ^[11] binding.”).

¹¹ Fcγ is the constant region of IgG involved in effector function. Specifically, “[t]he Fcγ binding site has been localized to the elbow region at the CH2 and CH3 interface of most IgG subclasses, and this binding property has been extensively used for the labeling and purification of antibodies.” Ex. 2013, 1. In other words, Fcγ and Fc terminology are used interchangeably in the art.

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Accordingly, we are not persuaded by Patent Owner’s contention that the art teaches away from the combination as articulated by Petitioner.

(4) Additional Modifications

We are also not persuaded by Patent Owner’s contention the ordinary artisan would not stop with a single G29A mutation in a SPA domain. *See* PO Resp. 43–45. Here, Abrahmsén expressly suggests making only a single mutation. Specifically, Abrahmsén contemplates “a recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon” without additional mutations. Ex. 1005, 2:33–37.

(5) Summary

Having considered the evidence and argument cited by the Petition, which we adopt as our own, we are persuaded that Petitioner has shown by a preponderance of evidence of record that the combination of Linhult and Abrahmsén teach each of the limitations of claims 1 and 14. Petitioner not only has articulated a sufficient motivation for making the combination but has also established that there is a reasonable expectation of success for the binding of an IgG antibody to a SPA domain that contains an G29A mutation.

b) Claims 4 and 17

Petitioner argues that “[t]he ‘capab[ility] of binding to the Fab part of an antibody,’ as recited in claims 4 and 17, is an inherent property of the claimed C(G29A)-based SPA ligand.” Pet. 28 (citing Ex. 1002 ¶¶ 120–127).

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Petitioner contends that a person of ordinary skill in the art did not need to recognize the Fab binding property of Domain C to be motivated to select that domain for modification. “A POSA would not have been motivated only by Fab-binding ability,[] as even Dr. Bracewell agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’” Reply 8 (citing Ex. 1057, 75:17–76:4, 113:23–114:11, 157:24–158:9; Ex. 1061 ¶ 29).

Patent Owner argues that “[n]either Linhult itself nor the Petition provide any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domain sequences (or in the figure, those plus Domain Z’s sequence). PO Resp. 29 (citing *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1337 (Fed. Cir. 2016)); *see also* Sur-reply 1 (“Petitioners fail to show that the person of ordinary skill in the art (the ‘POSA’) would have plucked Domain C from the sea of prior art teaching a preference for Domains B and Z, substituted one and only one amino acid—the glycine at position 29 with an alanine (a ‘G29A’ mutation)—and used that mutated protein as a chromatography ligand for the purification of antibodies or antibody fragments.”).

Patent Owner argues that

[t]he very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding ‘negligible’ when implemented in Domain B. Ex. 2009 at 2; *see also, e.g.*, Ex. 2010 at 2 (“Fab binding activity is located to a region determined by helices 2-3, including the position mutated to yield the Z domain.”); Ex. 2011 at 25 (“[I]t only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine to an alanine”); Ex. 2012 at 25-26 (“[D]omain

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Z containing a single G29A-substitution compared to domain B exhibits little or no [Fab] binding. This might be due to the substitution since the C_β of the alanine would perturb the interaction between the two molecules.”).

PO Resp. 40.

Claim 1 is directed to a composition. Claim 4 is dependent on claim 1 and further recites “wherein the ligand is capable of binding to the Fab part of an antibody.” Ex. 1001, 15:56–57. The “capable of binding” language of claim 4, however, does not add any structural limitations to claim 1 it merely recites the function of the composition when used for example in an assay.¹²

“[T]erms [that] merely set forth the intended use for, or a property inherent in, an otherwise old [or obvious] composition . . . do not differentiate the claimed composition from those known in the prior art.” *In re Pearson*, 494 F.2d 1399, 1403 (CCPA 1974). “Inherency may supply a missing claim limitation in an obviousness analysis. An inherent characteristic of a formulation [i.e. composition] can be part of the prior art in an obviousness analysis even if the inherent characteristic was unrecognized or unappreciated by a skilled artisan.” *Persion Pharms. LLC v. Alvogen Malta Operations Ltd.*, 945 F.3d 1184, 1190 (Fed. Cir. 2019) (citations omitted).

As explained above (II.E.5.a), Abrahmsén teaches replacing the glycine codon in the Asn-Gly constellation in *any one* the SPA domains with an alanine codon. Ex. 1005, 2:21–23. Abrahmsén further explains that:

The *Asn-Gly dipeptide* sequence is sensitive to hydroxylamine. As *this sequence is kept intact in all five IgG*

¹² The same issue applies to claims 14 and 17. We note that none of the claims in the ’765 patent are directed to a method of using the composition in an assay to isolate Fab fragments.

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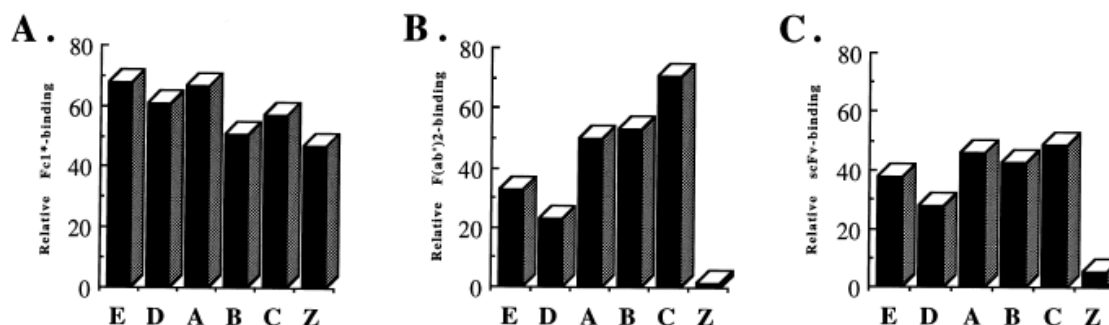
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binding domains of protein A and as this amino acid sequence is present in the middle of an alpha helix involved in the binding to IgG (FIG. 2) there is very little chance to be successful in any amino acid change. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.

Ex. 1005, 4:56–5:16 (emphasis added).

That the G29A mutation in domain B (resulting in domain Z) does not interfere with IgG binding was already established by Linhult. *See* Ex. 1004, 6 (Fig. 3 (showing IgG binding with domain Z)). The Domain Z binding property to IgG is further supported by Jansson. Jansson Fig. 3, reproduced below, shows the side-by-side comparison of Fc1*¹³, Fab, and scFv binding and confirms what was already suggested in Abrahmsén and Linhult – that a composition containing the G29A mutation in an SPA domain can bind IgG.



Panel A in Jansson Figure 3, reproduced above, shows that the single G29A mutation between Domain B and Domain Z does not result in the loss of IgG binding. Ex. 2029, 6 (Fig. 3). Because the other SPA domains are structurally so similar to Domain B there is a reasonable expectation that these domains would similarly retain the ability to bind IgG with the same G29A mutation. *See above* II.E.5.a.

¹³ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the IgG control in Jansson.

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We, therefore, agree with Petitioner that the limitation of the Fab binding ability as recited in claim 4 is an inherent feature of the structure disclosed in the independent claim 1. *See* Pet. 28 (citing Ex. 1002 ¶¶ 120–123). For the reasons disclosed above (II.E.5.a), we find that Petitioner has shown by a preponderance of evidence that there is a reasonable expectation that making a G29A mutation in *any one* of the SPA domains would result in a structure that retains the ability to bind IgG.

(1) Unexpected Results

Patent Owner argues that objective indicia of non-obviousness of the Domain C–G29A based ligands requires reaching a conclusion of non-obviousness. PO Resp. 53–55. Specifically, Patent Owner contends that “the SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” *Id.* at 55 (citing Ex. 2025 ¶ 123; Ex. 2030 at 18–19).

We are not persuaded by Patent Owner’s unexpected results argument. The prior art does not need to recognize that Domain C retains the ability to bind Fab fragments after a G29A mutation. Petitioner’s articulated rationale is that there is a reason to make the G29A mutations in *any one* of the SPA domains in order to get a product that is alkaline stable. Pet. 21–22 (citing Ex. 1002 ¶¶ 21, 99–11; Ex. 1004, 407–408, Fig. 1(a); Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). We find that Petitioner has shown by a preponderance of evidence that there is a reasonable expectation that making a G29A mutation in *any one* of the SPA domains results in a product that binds at least IgG. *See above* II.E.5.a. There is no requirement that the

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inherent characteristic of the Fab binding needed to be recognized in order to arrive at the conclusion that the recited structure in claim 1 would have been obvious. *See Persion Pharms.*, 945 F.3d at 1190 (“Our predecessor court similarly concluded that it ‘is not the law’ that ‘a structure suggested by the prior art, and, hence, potentially in the possession of the public, is patentable ... because it also possesses an [i]nherent, but hitherto unknown, function which [the patentees] claim to have discovered.’ *In re Wiseman*, 596 F.2d 1019, 1023 (C.C.P.A. 1979).”).

c) Claims 3 and 16

Claim 3 depends from claim 1, and claim 16 depends from claim 14. Claims 3 and 16 recite the additional limitation “wherein the chromatography matrix has retained at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.” Ex. 1001, 15:52–56. We find Petitioner has shown by a preponderance of the evidence that the combination of Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims for the reasons stated in the Petition which we adopt as our own. *See* Pet. 25–28, 30; Reply 20–21.

We are not persuaded by Patent Owner’s argument that “none of Petitioners’ cited references actually describe a C(G29A)-based SPA ligand, let alone provide alkaline stability data or test results concerning the same, the POSA is simply left to guess at how such a ligand would perform.” PO Resp. 49 (citing Ex. 2025 ¶¶ 121–124).

Petitioner has shown by a preponderance of the evidence of record that there is a reason for making the G29A mutation in *any one* of the four remaining SPA domains in order to produce a SPA product that is more

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alkaline stable and would reasonably bind IgG. We, therefore, agree with Petitioner, that the ability to retain the requisite binding capacity after a period of exposure to alkaline treatment is a property of the composition. Pet. 25–28; *see* Ex. 1002, ¶¶115–119; Ex. 1004, 6 (“Figure 3, the Z(N23T) mutant shows higher resistance to alkaline conditions than the Z domain when exposed to high pH values.”). That Linhult recognizes that additional mutations could further improve alkaline stability does not detract from Linhult’s teaching that a composition containing the single G29A mutation in SPA Domain B retains IgG binding. Ex. 1004, 6, *see id.* at 4 (“The Z-domain already possesses a significant tolerance to alkaline conditions.”).

d) Claims 2, 12, 15, and 25

Claims 2 and 12 depend from claim 1, and claims 15 and 25 depend from claim 14. We find Petitioner has shown by a preponderance of the evidence that the combination of Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims for the reasons stated in the Petition which we adopt as our own. *See* Pet. 25–28, 30.

We have reviewed Petitioner’s arguments and the underlying evidence cited in support and determine that Petitioner establishes that of Linhult, Abrahmsén, and Hober teaches the additional limitations of these dependent claims. Specifically, Petitioner asserts that Linhult describes multimerization of the ligand as recited in claims 2, 12, 15; and 25. Pet. 26 (citing Ex. 1004, 410; Ex. 1002 ¶¶112–114), *see id.* at 30 (citing Ex. 1002 ¶¶128–129).

Patent Owner does not offer arguments addressing Petitioner’s substantive showing with respect to claims 2, 12, 15, and 25. *See generally* PO Resp.

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6. *Summary*

For the foregoing reasons, we determine that Petitioner has shown by a preponderance of evidence that of claims 1–7, 10–20, and 23–26 of the ’765 patent are unpatentable based on the combination of Linhult, Abrahmsén, and Hober.

F. *Other Asserted Grounds*

Petitioner also asserts that claims 1–4, 12, 14–17, and 25 are unpatentable as obvious over Linhult and Abrahmsén (Pet. 18–30); claims 1–7, 10–20, and 23–26 are unpatentable as obvious over Linhult and Hober (*id.* at 30–48); claims 1–7, 10–20, 23–26 are unpatentable as obvious over Abrahmsén and Hober (*id.* at 49–60); claims 1–7, 10–20, and 23–26 are unpatentable over Berg and Linhult (’043 IPR Pet. 20–33); claims 2, 3, 15, and 16 are unpatentable over Berg, Linhult, and Hober (*id.* at 33–38); claims 1, 2, 5–7, 10–15, 18–20, and 23–26 are unpatentable over Berg and Abrahmsén (*id.* at 38–44); and claims 2–4, 15–17 are unpatentable over Berg, Abrahmsén, and Hober (*id.* at 44–46) under 35 U.S.C. §103(a). However, because Petitioner has already shown that the challenged claims 1–7, 10–20, and 23–26 are unpatentable over Linhult, Abrahmsén, and Hober as obvious, as discussed *supra*, we do not reach these additional asserted grounds. *See Beloit Corp. v. Valmet Oy*, 742 F.2d 1421, 1423 (Fed. Cir. 1984) (“The Commission . . . is at perfect liberty to reach a ‘no violation’ determination on a single dispositive issue.”); *Boston Sci. Scimed, Inc. v. Cook Grp., Inc.*, 809 F. App’x 984, 990 (Fed. Cir. 2020) (recognizing that “[t]he Board has the discretion to decline to decide additional instituted grounds once the petitioner has prevailed on all its challenged claims”).

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III. CONCLUSION¹⁴

For the foregoing reasons, we determine that Petitioner has demonstrated by a preponderance of the evidence that claims 1–7, 10–20, and 23–26 of the '765 patent are unpatentable on the bases set forth in the following table.

In summary:

Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–4, 12, 14–17, 25	103(a)	Linhult, Abrahmsén ¹⁵		
1–7, 10–20, 23–26	103(a)	Linhult, Hober ¹⁶		
1–7, 10–20, 23–26	103(a)	Linhult, Abrahmsén, Hober	1–7, 10–20, 23–26	
1–7, 10–20, 23–26	103(a)	Abrahmsén, Hober ¹⁷		

¹⁴ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this decision, we draw Patent Owner's attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. See 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. See 37 C.F.R. § 42.8(a)(3), (b)(2).

¹⁵ As explained above, we do not reach this '036 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

¹⁶ See *supra*, n.15.

¹⁷ See *supra*, n.15.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1-7, 10-20, 23-26	103(a)	Berg, Linhult ¹⁸		
2, 3, 15, 16	103(a)	Berg, Linhult, Hober ¹⁹		
1, 2, 5-7, 10-15, 18-20, 23-26	103(a)	Berg, Abrahmsén ²⁰		
2-4, 15-17	103(a)	Berg, Abrahmsén, Hober ²¹		
Overall Outcome			1-7, 10-20, 23-26	

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the preponderance of the evidence of record has shown that claims 1-7, 10-20, and 23-26 of the '765 patent are found unpatentable; and

FURTHER ORDERED because this is a final written decision, the parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

¹⁸ As explained above, we do not reach this '043 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

¹⁹ See *supra*, n.18.

²⁰ See *supra*, n.18.

²¹ See *supra*, n.18.

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Paper 40
Date: May 18, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

JSR CORPORATION and JSR LIFE SCIENCES, LLC,
Petitioner,

v.

CYTIVA BIOPROCESS R&D AB,
Patent Owner.

IPR2022-00041
IPR2022-00044
Patent 10,343,142 B2

Before ULRIKE W. JENKS, SHERIDAN K. SNEDDEN, and
SUSAN L. C. MITCHELL, *Administrative Patent Judges*.

JENKS, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining Some Challenged Claims Unpatentable
35 U.S.C. § 318

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IPR2022-00044
Patent 10,343,142 B2

I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review of claims 1–7, 10–20, and 23–30 (“the challenged claims”) of U.S. Patent No. 10,343,142 B2 (Ex. 1001, “the ’142 patent”). We have jurisdiction under 35 U.S.C. § 6, and enter this Final Written Decision pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons set forth below, we determine that JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) has shown, by a preponderance of the evidence, that some of the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e).

A. *Consolidated Proceedings*

The two captioned proceedings (IPR2022-00041 and IPR2022-00044 (or “the ’044 IPR”)) involve the ’142 patent and challenge the same set of claims. The asserted grounds and prior art contentions are different in each proceeding. Consolidation is appropriate where, as here, the Board can more efficiently handle the common issues and evidence, and also remain consistent across proceedings. Under 35 U.S.C. § 315(d), the Director may determine the manner in which these pending proceedings may proceed, including “providing for stay, transfer, consolidation, or termination of any such matter or proceeding.” *See also* 37 C.F.R. § 42.4(a) (“The Board institutes the trial on behalf of the Director.”). There is no specific Board rule that governs consolidation of cases. Rule 42.5(a), however, allows the Board to determine a proper course of conduct in a proceeding for any situation not specifically covered by the rules and to enter non-final orders to administer the proceeding. *See* 37 C.F.R. § 42.5(a).

Therefore, on behalf of the Director under § 315(d), and for a more efficient administration of these proceedings, we consolidate IPR2022-

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00041 and IPR2022-00044 for purposes of rendering this Final Written Decision.

B. *Evidence*

Petitioner relies upon information that includes the following:

Ex. 1004, M. Linhult, *et al.*, *Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach*, 55 PROTEINS: STRUCTURE, FUNCTION, AND BIOINF., 407–16 (2004) (“Linhult”).

Ex. 1005, L. Abrahmsén, *et al.*, U.S. Patent No. 5,143,844 (issued Sept. 1, 1992) (“Abrahmsén”).

Ex. 1006, S. Hober, PCT Publication No. WO 03/080655 A1 (published Oct. 2, 2003) (“Hober”).

C. *Procedural History*

Petitioner filed a Petition for an *inter partes* review of the challenged claims under 35 U.S.C. § 311. Paper 1¹ (“Pet.”). Petitioner supported the Petition with the Declaration of Dr. Steven M. Cramer. Ex. 1002. Cytiva Bioprocess R&D AB (“Patent Owner”) filed a Patent Owner Preliminary Response to the Petition. Paper 8.

On May 19, 2022, pursuant to 35 U.S.C. § 314(a), we instituted trial (“Decision” or “Dec.” (Paper 9)) to determine whether any challenged claim of the ’142 patent is unpatentable.

¹ We note that the evidence filed in both proceedings is generally consistent in having the same exhibit number. Therefore, we reference exhibits and paper numbers as they appear in the record of IPR2022-00041, unless otherwise noted.

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In IPR2022-00041, Petitioner asserts the following grounds of unpatentability (Pet. 4):

Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–4, 12, 14–17, 25, 27–30	103(a)	Linhult, Abrahmsén
1–7, 10–20, 23–30	103(a)	Linhult, Hober
1–7, 10–20, 23–30	103(a)	Linhult, Abrahmsén, Hober
1–7, 10–20, 23–30	103(a)	Abrahmsén, Hober

In IPR2022-00044, Petitioner asserts the following grounds of unpatentability (’044 IPR Pet. 4):

Claim(s) Challenged	35 U.S.C. §	Reference(s)/Basis
1–7, 10–20, 23–26	103(a)	Berg, Linhult
2, 3, 15, 16	103(a)	Berg, Linhult, Hober
1, 2, 5–7, 10–15, 18–20, 23–26	103(a)	Berg, Abrahmsén
2–4, 15–17, 27–30	103(a)	Berg, Abrahmsén, Hober

Patent Owner filed a Patent Owner Response to the Petition. Paper 15 (“PO Resp.”). Patent Owner supported the Response with the Declaration of Dr. Daniel Bracewell (Ex. 2025). *See* PO Resp., iv (Exhibit List). Petitioner filed a Reply to the Patent Owner Response. Paper 28 (“Reply”). Petitioner

² The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the ’142 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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supported the Reply with a Reply Declaration from Dr. Steven M. Cramer. Ex. 1061. Patent Owner filed a Sur-reply to Petitioner's Reply. Paper 34 ("Sur-reply").

On February 16, 2023, the parties presented arguments at an oral hearing. Paper 35. The hearing transcript has been entered in the record. Paper 39 ("Tr.").

For the reasons set forth below, we determine that Petitioner has shown by a preponderance of the evidence that claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the '142 patent are unpatentable, but find that Petitioner has not shown by a preponderance of the evidence that claims 4 and 17 are unpatentable.

D. *Real Parties in Interest*

Petitioner identifies itself, JSR Corporation and JSR Life Sciences, LLC, along with JSR Micro NV, as the real parties-in-interest. Pet. 2. Patent Owner identifies itself, Cytiva Bioprocess R&D AB, along with Cytiva Sweden AB and Danaher Corporation as real parties-in-interest. Paper 5, 1.

E. *Related Matters*

The '142 patent is at issue in *Cytiva BioProcess R&D et al. v. JSR Corp. et al.*, Civil Action No. 21-310-RGA (D. Del.). Pet. 2; Paper 5, 1.

In addition to the '142 patent challenged here, Petitioner has filed Petitions for *inter partes* review of related U.S. patents as follows: U.S. Patent No. 10,213,765 B2 ("the '765 patent") in IPR2022-00036 and IPR2022-00043; and U.S. Patent No. 10,875,007 B2 ("the '007 patent") in IPR2022-00042 and IPR2022-00045. Pet. 2–3; Paper 5, 1–2. Petitioner indicates that the '765 patent and the '007 patent are also being asserted in

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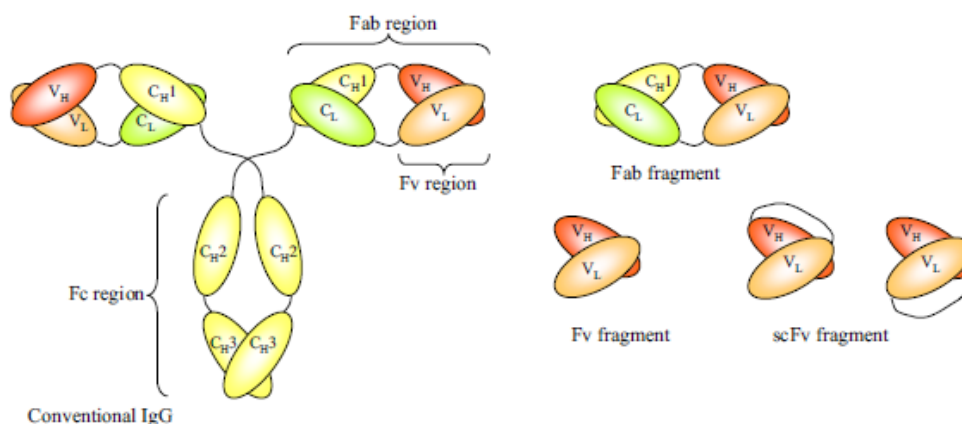
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the above-cited district court case. Pet. 3. The parties further list a pending application in the same family, U.S. App. Serial No. 17/107,600. Pet. 2; Paper 5, 2.

F. *Subject matter background*

Antibodies (also called immunoglobulins) are glycoproteins, which specifically recognize foreign molecules. These recognized foreign molecules are called antigens. Ex. 2001, 1. A schematic representation of the structure of a conventional IgG and fragments is shown below:



The figure (Ex. 2001, 2 (Fig. 1)), reproduced above, shows

the structure of a conventional IgG and fragments that can be generated thereof. The constant heavy-chain domains CH1, CH2 and CH3 are shown in yellow, the constant light-chain domain (CL) in green and the variable heavy-chain (VH) or light-chain (VL) domains in red and orange, respectively. The antigen binding domains of a conventional antibody are Fabs and Fv fragments. Fab fragments can be generated by papain digestion. Fvs are the smallest fragments with an intact antigen-binding domain. They can be generated by enzymatic approaches or expression of the relevant gene fragments (the recombinant version). In the recombinant single-chain Fv fragment, the variable domains are joined by a peptide linker. Both possible configurations of the variable domains are shown, i.e. the carboxyl terminus of VH fused to the N-terminus of VL and vice

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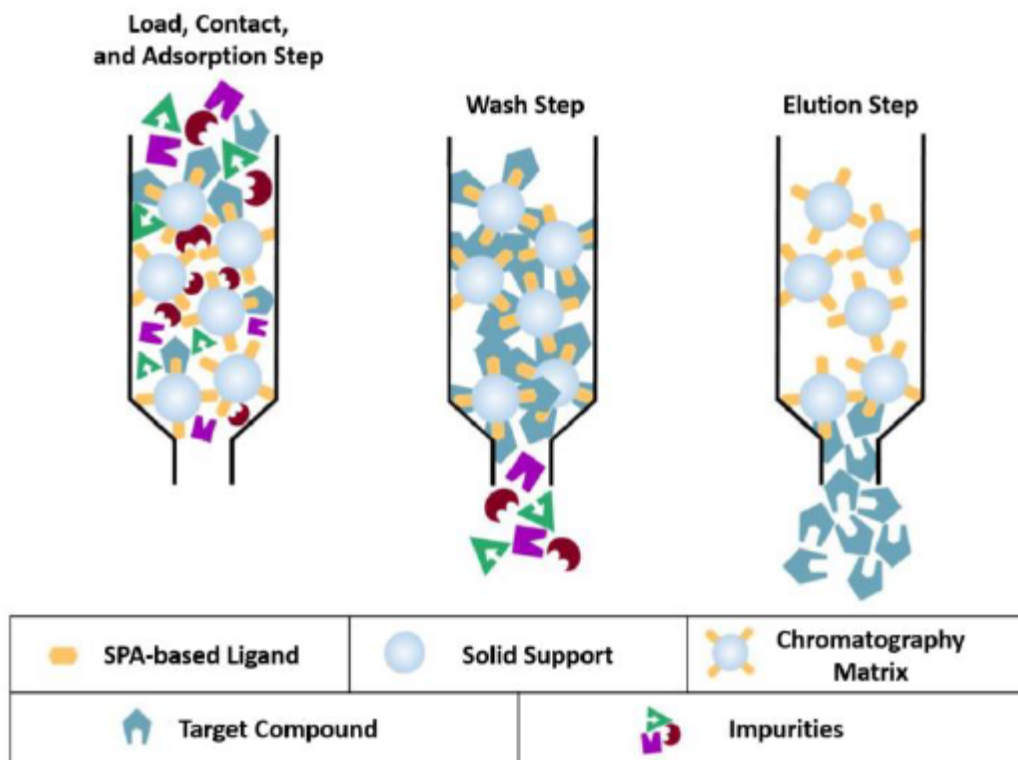
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versa.

Ex. 2001, 2; *see also* PO Resp. 5.

Below is a generic, exemplary schematic that shows how affinity purification typically works:



The figure shows the schematic of the loading, contact, and adsorbing step onto a column, followed by the wash step, and finally the elution and collection of the target compound. Ex. 1002 ¶ 24 (citing Ex. 1014 §§ 1.1, 4.2.); *see also* PO Resp. 7 (“In a typical process, the composition containing the desired antibody then is loaded onto (i.e., pumped or injected into) the column.”); Pet. 6; *see generally* Ex. 1014.

G. *The '142 patent (Ex. 1001)*

The '142 patent is titled “Chromatography Ligand Comprising Domain C from *Staphylococcus Aureus* Protein A for Antibody Isolation.”

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Ex. 1001, (54). The '142 patent relates to an affinity ligand that is used for antibody isolation. *Id.* at 1:39–41. The '142 patent explains that chromatography is used in large-scale economic production of drugs and diagnostics in which proteins are produced by cell culture and then separated from the mixture of compounds and other cellular components to a sufficient purity. *Id.* at 1:52–61. One type of chromatography matrix for this purifying process includes immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG). *Id.* at 2:4–13. The '142 patent further explains that “[a]s in all process technology, an important aim is to keep the production costs low” by reusing matrices via cleaning protocols such as an alkaline protocol known as Cleaning In Place (CIP). *Id.* at 2:14–29. However, harsh treatments may impair the chromatography matrix materials such that there is a need for stability towards alkaline conditions for an engineered protein ligand. *Id.* at 2:31–48.

The '142 patent discloses that Protein A, known as SpA, is a constituent of the cell wall of the bacterium *Staphylococcus aureus*, and is widely used as a ligand in affinity chromatography matrices due to its ability to bind with IgG. *Id.* at 2:49–54. SpA is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind to antibodies at the Fc region, and it has been shown that each of these domains binds to certain antibodies at the Fab region. *Id.* at 2:54–63.

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Domain C from SpA is defined by SEQ ID NO: 1 and is reproduced below.

```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1           5           10           15

Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
          20           25           30

Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
          35           40           45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50           55

```

Id. at 4:26 (highlighting added by Board); 15:1–38; *see also* Pet. 9. SEQ ID NO: 1 shows domain C has Glycine (Gly) as an amino acid at the position 29 as annotated via red highlighting. According to the '142 patent, it has already been shown “that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A” and the '142 patent discloses that from experiments, “the present inventors have quite surprisingly shown that the SpA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product.” Ex. 1001, 5:38–40, 51–55. The '142 patent discloses, “it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences -asparagine-glycine- and -asparagine-serine.” *Id.* at 5:62–66. The '142 patent then discloses “[q]uite surprisingly, the Domain C ligand of the invention presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29” and “[t]hus, in a specific embodiment, the chromatography ligand according to the invention comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A.” *Id.* at 5:67–6:3, 6:49–52. The '142 patent discloses that a multimeric chromatography ligand

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(also denoted a “multimer”) can be comprised of at least two domain C units and that a chromatography matrix can be comprised of ligands coupled to an insoluble carrier. *Id.* at 7:27–29, 8:21–23. The ’142 patent discloses a column study of alkaline stability of its Protein A-derived ligands and a testing of the Fab-binding of its ligands. *Id.* at 10:32–14:59. The study includes using an injection liquid and solution along with human normal immunoglobulin as a target compound in chromatography experiments, ligand coupling and column packing, adsorbance measurements, washing out unbound samples, and eluting bound material. *Id.* at 11:11–18, 12:25–34, 13:32–37.

1. Illustrative Claim

Claims 1 and 14 are the independent claims challenged by Petitioner in this proceeding. Independent claim 1, reproduced below, is illustrative of the subject matter:

1. A process for isolating one or more target compound(s), the process comprising:

(a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:

(i) a solid support; and

(ii) at least one ligand coupled to the solid support, the ligand comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1; and

(b) adsorbing the target compound(s) to the ligand; and,

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(c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand.

Ex. 1001, 15:40–56. Claim 14 is similar to claim 1 but recites “a ligand” rather than “at least one ligand,” and wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids “in alignment with SEQ ID NO. 1” rather than “contiguous amino acids of a modified SEQ ID NO. 1.” *Id.* at 16:57–17:6.

II. ANALYSIS

A. *Principles of Law*

“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify “with particularity . . . the evidence that supports the grounds for the challenge to each claim”)). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner must demonstrate by a preponderance of the evidence³ that the claims are unpatentable. 35 U.S.C. § 316(e); 37 C.F.R. § 42.1(d). A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter,

³ The burden of showing something by a preponderance of the evidence requires the trier of fact to believe that the existence of a fact is more probable than its nonexistence before the trier of fact may find in favor of the party who carries the burden. *Concrete Pipe & Prods. of Cal., Inc. v. Constr. Laborers Pension Tr. for S. Cal.*, 508 U.S. 602, 622 (1993).

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as a whole, would have been obvious at the time of the invention to a person having ordinary skill in the art. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations, including “the scope and content of the prior art”; “differences between the prior art and the claims at issue”; “the level of ordinary skill in the art;” and any “objective evidence of non-obviousness.” *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. *Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the

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technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner asserts that a person of ordinary skill in the art would have had

(1) at least an advanced degree (*e.g.*, a Master’s or Ph.D.) in biochemistry, process chemistry, protein chemistry, chemical engineering, molecular and structural biology, biochemical engineering, or similar disciplines; (2) several years of post-graduate training or related experience (including industry experience) in one or more of these areas; and (3) an understanding of the various factors involved in purifying proteins using chromatography.[] Such a person would have had multiple years of experience with affinity ligand design and protein purification.

Pet. 9–10 (citing Ex. 1002 ¶¶ 13–14). Patent Owner does not dispute Petitioner’s definition of the person of ordinary skill. *See generally* PO Resp. Because Petitioner’s proposed definition is unopposed and appears consistent with the Specification and art of record, we apply it here.

C. *Claim Construction*

The Board applies the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.200(b) (2021). Under that standard, claim terms “are generally given their ordinary and customary meaning” as understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc).

Petitioner contends that based on Patent Owner’s implicit construction in the district court litigation “that the term ‘the ligand comprising at least

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two polypeptides’ refers to a multimeric ligand (such as a tetramer) comprised of multiple polypeptides, each of which is a monomer.” Pet. 15 (citing Ex. 1020 ¶¶ 41, 50, 58, 62, 74, 87).

Patent Owner does not contest Petitioner’s construction. *See generally* PO Resp.

According to the Specification, “the present invention . . . relates to a multimeric chromatography ligand (also denoted a ‘multimer’) comprised of at least two Domain C units, or a functional fragments or variants thereof.” Ex. 1001, 7:27–30. The Specification additionally recites that a multimer containing only domain C units can, however, include linkers. *Id.* 7:39–41. In addition, the Specification describes that “the multimer comprises one or more additional units, which are different from Domain C.” *Id.* 7: 44–45. Based on these disclosures in the Specification, a multimer is composed of at least two or more monomers.

Because Petitioner’s construction is consistent with the ’142 patent’s express construction of the term, and because Patent Owner agrees with Petitioner’s construction, we apply it here.

D. *Overview of Asserted References*

1. *Linhult (Ex. 1004)*

Linhult is titled “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach.” Ex. 1004, 1. Linhult discloses that due to the high affinity and selectivity of Staphylococcal protein A (SPA), “it has a widespread use as an affinity ligand for capture and purification of antibodies,” but that “it is desirable to further improve the stability in order to enable an SPA-based affinity medium to withstand even longer exposure to the harsh conditions

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associated with cleaning-in-place (CIP) procedures.” *Id.*, Abstr. Linhult discloses, “[t]o further increase the alkaline tolerance of SPA, we chose to work with Z, which is a small protein derived from the B domain of SPA.” *Id.* at 2.

Figures 1A and 1B of Linhult are reproduced below.

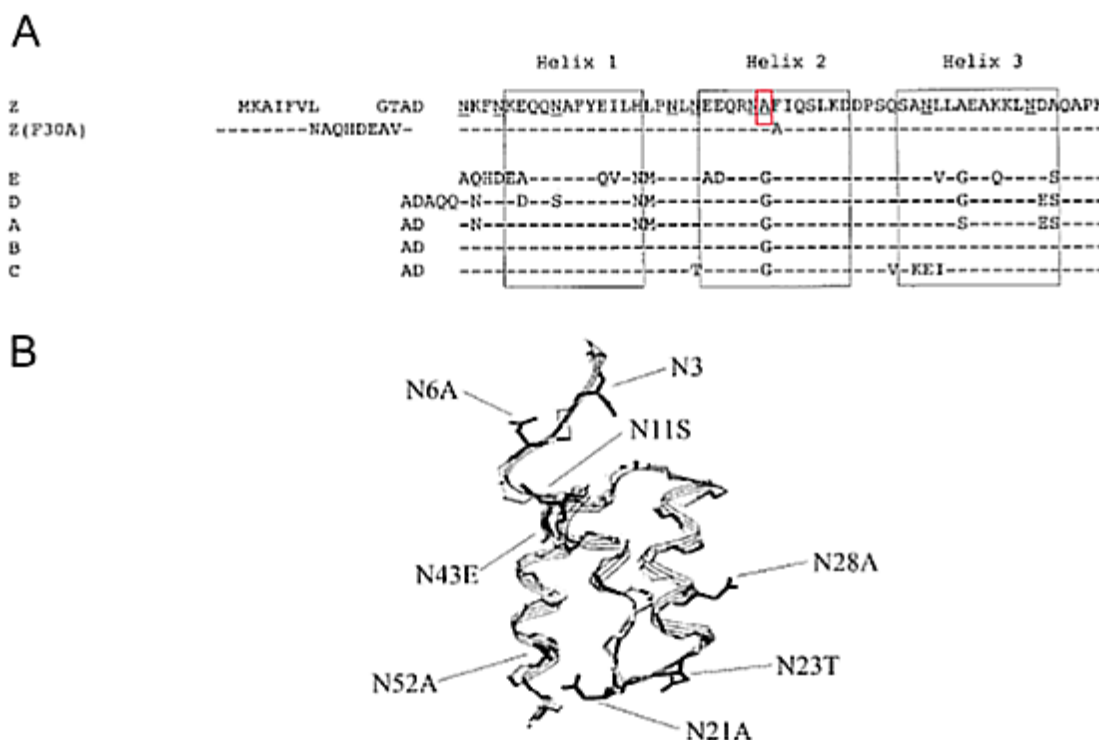


Figure 1A shows “[a]mino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C)” in which the horizontal lines indicate amino acid identity and “one glycine in the B domain [is] replaced [and] underlined” as annotated by the Board with a red box. *Id.* “Z(F30A), and all mutants thereof includes the same N-terminal as Z(F30A)” and

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“Z(N23T) was constructed with the same N-terminal as Z.” *Id.*⁴ Figure 1B shows “[t]he three-dimensional structure of the Z domain” and “the different substitutions are indicated.” *Id.* Specifically, Linhult discloses,

[t]he B domain has been mutated in order to achieve a purification domain resistant to cleavage by hydroxylamine. An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine.[] Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.[] Protein Z is well characterized and extensively used as both ligand and fusion partner in a variety of affinity chromatography systems.

Id. Using a 0.5 M NaOH cleaning agent and “a total exposure time of 7.5 h for Z(F30A) and mutants thereof,” Linhult determines that “N23 seems to be very important for the functional stability after alkaline treatment of Z(F30A)” and “Z(F30A, N23T) shows only a 28% decrease in capacity despite the destabilizing F30A-mutation.” *Id.* at 410–11; Figs. 2, 3. Linhult reports that “[h]ence, the Z(F30A, N23T) is almost as tolerant as Z and is thereby the most improved variant with Z(F30A) as scaffold.” *Id.* at 411; Figs. 2, 3.

Linhult further discloses that “Z, Z(F30A), and mutated variants were covalently coupled to HiTrap™ affinity columns,” that “[t]he Z domain includes 8 asparagines (N3, N6, N11, N21, N23, N28, N43, and N52; Fig. 1),” and that “since the amino acid is located outside the structured part of the domain, it will most likely be easily replaceable during a multimerization of the domain to achieve a protein A–like molecule.” *Id.* at 410. Linhult

⁴ The mutation N23T having a change in amino acid correlates with the amino acid N next to the “Helix 2” box of Figure 1A as annotated by Petitioner. *See* Pet. 12.

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confirms that “the affinity between Z(F30A) and IgG was retained despite the mutation.” *Id.* In Linhult’s studies, “[h]uman polyclonal IgG in TST was prepared and injected onto the columns in excess” and “[a] standard affinity chromatography protocol was followed.” *Id.*

2. *Abrahmsén (Ex. 1005)*

Abrahmsén “relates to a recombinant DNA fragment coding for an immunoglobulin G ([I]gG) binding domain related to staphylococcal protein A . . . and to a process for cleavage of a fused protein expressed by using such fragment or sequence.” Ex. 1005, 1:8–13. Abrahmsén discloses that “[b]y making a gene fusion to staphylococcal protein A any gene product can be purified as a fusion protein to protein A and can thus be purified in a single step using IgG affinity chromatography.” *Id.* at 1:22–26. Abrahmsén explains that Protein A has “5 Asn-Gly in the IgG binding region of protein A” which “makes the second passage through the column irrelevant as the protein A pieces released from the cleavage will not bind to the IgG.” *Id.* at 1:58–63. Abrahmsén provides a solution to this problem “by adapting an IgG binding domain so that no Met and optionally no Asn-Gly is present in the sequence.” *Id.* at 1:64–67.

Abrahmsén discloses that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén discloses that the recombinant DNA fragment can “cod[e] for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the

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Asn-Gly coding constellation has been replaced by an alanine codon.” *Id.* at 2:32–37. According to Abrahmsén, from a simulation of the Gly to Ala amino acid change in the computer, it was “concluded that this change would not interfere with folding to protein A or binding to IgG.” *Id.* at 5:13–16.

3. *Hober (Ex. 1006)*

Hober “relates to . . . a mutant protein that exhibits improved stability compared to the parental molecule” and “also relates to an affinity separation matrix, wherein a mutant protein according to the invention is used as an affinity ligand.” Ex. 1006, 1. Hober discloses that removal of contaminants from the separation matrix involves “a procedure known as cleaning-in-place (CIP)” but “[f]or many affinity chromatography matrices containing proteinaceous affinity ligands,” the alkaline environment “is a very harsh condition and consequently results in decreased capacities owing to instability of the ligand.” *Id.* at 1–2. According to Hober, stability to alkaline conditions can be engineered into a protein. *Id.* at 2. To improve the stability of a Streptococcal albumin-binding domain (ABD) in alkaline environments, it has been reported to involve the role of peptide conformation in the rate and mechanism of deamidation of asparaginyll residues and that “the shortest deamidation half time have been associated with the sequences -asparagine-glycine and -asparagine-serine.” *Id.* at 2. Further, from a study of a mutant of ABD that was created, it was concluded that “all four asparagine residues can be replaced without any significant effect on structure and function.” *Id.* at 2–3. Hober points out that the staphylococcal protein A (SPA) contains domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species and

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reagents of this protein with their high affinity and selectivity have found a widespread use in the field of biotechnology. *Id.* at 3. Accordingly, “there is a need in this field to obtain protein ligands capable of binding immunoglobulins, especially via the Fc-fragments thereof, which are also tolerant to one or more cleaning procedures using alkaline agents.” *Id.* at 4.

In one embodiment of Hober, a multimer “comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A” in which “asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids” for advantageous structural stability reasons wherein “the glycine residue in position 29 of SEQ ID NO: 1 has also been mutated, preferably to, an alanine residue.” *Id.* at 12. Hober’s SEQ ID NO: 1 and is reproduced below.

```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
 1          5          10          15

Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln
          20          25          30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
          35          40          45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
 50          55

```

Id. at SEQUENCE LISTING 1. SEQ ID NO: 1 shows a domain of *Staphylococcus aureus* having Glycine (Gly) as an amino acid at the position 29, as annotated by the Board via red highlighting.

Hober further discloses that its matrix for affinity separation “comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which [in the] protein at least one asparagine residue has been mutated to an amino acid other than glutamine.” *Id.* at 13. For its method of isolating an immunoglobulin, Hober discloses “in a first step, a

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solution comprising the target compounds, . . . is passed over a separation matrix under conditions allowing adsorption of the target compound to ligands present on said matrix” and “[i]n a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption, i.e. release of the target compound.” *Id.* at 13.

E. *Obviousness in view of Linhult, Abrahmsén, and Hober*

1. *Petitioner’s Contentions*

a) *Claims 1 and 14*

Petitioner contends that “*Linhult* describes the common use of chromatography matrices in the biotechnology field, and, more specifically, SPA-based chromatography matrices to isolate target compounds.” Pet. 16 (citing Ex. 1002 ¶ 83). Petitioner contends that “*Linhult* describes a process whereby a ‘[h]uman polyclonal IgG in TST^[5] was prepared and injected onto the columns in excess,’ ‘[a] standard affinity chromatography protocol was followed,’ and ‘eluted material was detected.’” Pet. 17 (citing Ex. 1004, 4). Petitioner contends that a person of ordinary skill in the art “would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of loading a liquid comprising the target compound onto the column, thereby allowing the liquid to contact the recited SPA-based chromatography matrix.” *Id.* (citing Ex. 1002 ¶¶ 88–89). In other words, Petitioner contends that the contacting step is a well-known step in the field of affinity chromatography.

⁵ TST is a solution containing 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20. Ex. 1004, 4.

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Petitioner contends that “[a]dsorbing target compounds to SPA-based ligands coupled to the solid supports was a well-known and conventional feature of SPA-based affinity chromatography.” Pet. 23 (citing Ex. 1002 ¶ 119, *see also id.* ¶¶ 120–121; Ex. 1004, 4).

Petitioner contends that “a [person of ordinary skill in the art] would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of eluting target compounds from SPA-based ligands coupled to the solid supports in a chromatography matrix.” Pet. 24 (citing Ex. 1002 ¶ 126, *see also id.* ¶¶ 123–127; Ex. 1004 ¶ 4). Petitioner, therefore contends that it is well-known that a standard affinity chromatography protocol contains three active steps: (1) contacting, (2) adsorbing, and (3) eluting.

Petitioner contends that Linhult teaches a chromatography matrix. Pet. 18. Specifically, Linhult teaches using a HiTrap chromatography affinity column made up of agarose beads that serve as a solid support for “coupling SPA-based ligands.” Pet. 18 (citing Ex. 1004, 4; Ex. 1002 ¶ 95). “*Linhult* discloses that its SPA-based ligands were ‘coupled to’ the solid support agarose beads [] contained in HiTrap™ affinity columns.” *Id.* at 18 (citing Ex. 1004, 4). Petitioner contends that “Linhult discloses that ‘multimerization’ of SPA monomers is performed to ‘achieve’ an ‘[SPA-]like’ affinity ligand.” *Id.* at 19 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 100–103). Petitioner contends that “Figure 1(a), *Linhult* describes at least 55 amino acids of SPA’s naturally-occurring C domain (i.e., SEQ ID NO. 1).” *Id.* (citing Ex. 1004, 1, Fig. 1(a); *see* Ex. 1005, Fig. 2; Ex. 1006, Fig. 1; Ex. 1008, 639, Fig. 1). Petitioner contends “that all ‘five SPA domains show individual affinity for the Fc-fragment . . . as well as certain Fab-fragments

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of [antibodies] from most mammalian species.” *Id.* at 20 (citing Ex. 1004, 1).

Petitioner contends that it was “known that individual SPA domains, including the C domain, could be used to construct SPA-based affinity ligands for purifying proteins.” *Id.* at 20 (citing Ex. 1002 ¶¶ 29, 101; Ex. 1004, 1; Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). Petitioner contends that Linhult teaches a person of ordinary skill in the art “that avoiding the Asn₂₈-Gly₂₉ dipeptide sequence through a G29A mutation, including on the C domain, would yield an SPA-based ligand having increased alkali-stability.” *Id.* at 21 (citing 1002 ¶¶ 109–113; Ex. 1011; Ex. 1012; Ex. 1013). Petitioner acknowledges that “*Linhult* does not expressly disclose a C(G29A)-based SPA ligand,” but asserts that “[r]egardless, it would have been obvious to a [person of ordinary skill in the art] to modify *Linhult* based on the teachings of *Abrahmsén* to incorporate a C(G29A)-based SPA ligand in a chromatography matrix.” *Id.* at 23 (Ex. 1002 ¶¶ 108–117). Petitioner contends “*Abrahmsén* expressly discloses ‘a recombinant DNA coding for **any of** the E D A B C domains of [SPA], wherein the glycine codon(s) in the Asn_[28]-Gly_[29] coding constellation has been replaced by an alanine codon.’” *Id.* at 22 (bracketing in and emphasis in original) (citing Ex. 1005, 2:32–37).

A [person of ordinary skill in the art] would have had good reason to combine the teachings from *Abrahmsén* with *Linhult* because a G29A mutation was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence, i.e., the “most sensitive amino acid sequence to alkaline conditions,” such as those used in CIP. (Ex. 1002 ¶¶ 116, 129; Ex. 1004, [2].) Moreover, a [person of ordinary skill in the art] would have been drawn to a C-domain-based ligand, which, as *Linhult* describes, shows individual affinity for antibodies and

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already includes 23T (as well as 43E), which it disclosed as providing “remarkably increased” stability. (Ex. 1002 ¶¶112-13; Ex. 1004, [1], [8–9].)

Pet. 22–23.

[A]pplying the teachings of *Abrahmsén* with *Linhult* would have involved merely combining known elements in the field (e.g., a process for isolating one or more target compounds using an affinity chromatography matrix comprising a G29A-containing ligand coupled to a solid support, as in *Linhult*, and a C(G29A)-based amino acid sequence, as in *Abrahmsén*) according to known ligand-construction methods to yield a predictable results (e.g., a process for isolating one or more target compounds using the recited affinity chromatography matrix). (Ex. 1002 ¶130.) See, e.g., *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 415-21 (2007); *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1239-40 (Fed. Cir. 2010).

Pet. 24–25. Petitioner further contends “*Hober*’s disclosure is in the context of SPA-based affinity chromatography utilizing G29A-containing ligands, and, in fact, further confirms that the teachings of *Abrahmsén* are applicable in this context.” Pet. 48 (citing Ex. 1002 ¶¶ 236–250; Ex. 1006, 10–12); Ex. 1006, 12 (“the present multimer also comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A. . . . for structural stability reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to an alanine residue”).

b) Claims 4 and 17

Petitioner contends that “[t]he capability of “bind[ing] to the Fab part of an antibody,” as recited in claims 4 and 17, is an inherent property of the recited C(G29A)-based SPA ligand.” Pet. 28 (citing Ex. 1002 ¶¶ 141–148).

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c) Claims 2, 3, 12, 15, 16, 25, and 27–30

With respect to claims 2, 3, 12, 15, 16, 25, and 27–30, Petitioner directs our attention to where in the asserted art of record the various limitations of the dependent claims may be found. *See* Pet. 26–28, 30–31.

2. Patent Owner’s Contentions

Patent Owner argues that the Petition fails to demonstrate that there would have been motivation and a reason to make and use the chromatography matrix as claimed (PO Resp. 17–38) for the following reasons: that the Petition has not established that there is a reasonable expectation of success in arriving at the claimed matrix (*id.* at 48–54); that the art teaches away from making the G29A modification (*id.* at 38–44); and that the artisan would have been motivated to make alternative mutations than the claimed ones (*id.* at 44–48).

a) Matrix

According to Patent Owner, “Petitioners fail to explain *why* the POSA would have been motivated to select Domain C’s amino acid sequence as the foundation for an engineered SPA ligand with favorable properties.”

PO Resp. 18. Specifically arguing that the obviousness analysis requires the prior art be viewed as a whole. PO Resp. 20 (citing *In re Wesslau*, 353 F.2d 238 (CCPA 1965); *In re Enhanced Sec. Rsch., LLC*, 739 F.3d 1347, 1355 (Fed. Cir. 2014); *Impax Lab’s Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018)). In other words, because obviousness requires considering the prior art as a whole and no one was working on domain C at the time of the invention, Patent Owner asserts, it would not have been obvious to select domain C for further development or genetic modification.

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Patent Owner argues that because nobody was working on domain C at the time the invention was filed, the selection of domain C for further development could not possibly be obvious. *See* PO Resp. 23 (“Reliance on *KSR* also is foreclosed by the evidence that no one in the art was seeking to modify Domain C.”), *see also id.* at 24 (“But no prior art cited by Petitioners singles Domain C out for further development. Ex. 2025 ¶¶ 89–96”), *id.* at 26–27 (“Dr. Cramer [Petitioner’s expert] himself highlights, it would have been natural for the POSA to further develop the domain—Domain B—that was best understood and for which there was a crystal structure available. Ex. 1002 ¶ 33; Ex. 2015 at 137:20–138:19; Ex. 2017”), *id.* at 28 (“The notion that this body of work would lead the POSA to discard the improved ligands the references themselves focus on, and instead start experimenting with mutations to Domain C—strains credulity. Ex. 2025 ¶¶ 92–95”).

According to Patent Owner, neither Linhult nor Abrahmsén supply the motivation to start with domain C. “Linhult focuses exclusively on, and concerns improvements to, the alkaline stability of Domain Z by mutating asparagine residues. *See* Ex. 2025 ¶¶ 64–67, 92.” PO Resp. 29. “Rather than use Domain C, the POSA reviewing Linhult would be motivated to keep working with Domain Z, adopting the N23T mutation. Ex. 2025 ¶ 67 & n.3.” PO Resp. 30. “Neither Abrahmsén itself nor the Petition provide[s] any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domains. *WBIP[LLC v. Kohler Co.]*, 829 F.3d 1317, 1337 (Fed. Cir. 2016)].” PO Resp. 31.

b) Reasonable Expectation of Success

Patent Owner argues that “the field of protein engineering is notoriously unpredictable.” PO Resp. 22 (citing Ex. 2025 ¶¶ 50–52).

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Arguing that “despite their supposed structural similarity, there are a number of differences between the naturally-occurring domains of protein A, including five different amino acids in the sequences of Domain B (with which the industry was quite familiar) and Domain C (which remained virtually ignored as of the priority date).” *Id.* at 22–23 (citing Ex. 2025 ¶ 48).

Protein engineering is a highly complex and unpredictable field and was all the more so as of the priority date more than fifteen years ago. *See, e.g.,* Ex. 2025 ¶¶ 50-52. . . . As amply demonstrated by the effect of the G29A mutation on Domain Z’s Fab-binding ability, even a single amino acid substitution can drastically alter the properties of a protein. Ex. 2025 ¶ 52; Ex. 2015 at 51:15-52:1 ([Dr. Cramer, Petitioner’s expert] agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability), 18:10-12, 73:16-20.

PO Resp. 35–36.

Patent Owner argues that

The Federal Circuit has rejected arguments premised on the notion that a homologous structure renders an invention obvious, particularly given the difficulty and uncertainty in the art as of the priority date. *See, e.g., Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208 (Fed. Cir. 1991) (holding the use of a monkey gene to probe for a roughly 90 percent “homologous” human gene would not have been obvious, particularly given expert testimony that isolating a particular gene would have been “difficult” and the lack of certainty in the endeavor).

PO Resp. 37. Specifically, Patent Owner argues that the Fab-binding capability of a ligand could not have been predicted, and therefore, there is no reasonable expectation of success in using the claimed ligand in a process of purifying a target compound. *See* PO Resp. 48–53.

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c) Teaching Away

Patent Owner argues that the prior art would have told the person of ordinary skill in the art to avoid a G29A a modification to domain C.

PO Resp. 39. In other words, its Patent Owner's contention that the prior art teaches away from making this modification. "The very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding 'negligible' when implemented in Domain B." PO Resp. 41. Patent Owner argues that a person seeking to improve Fab binding would have avoided a G29A substitution of domain C. PO Resp. 41–44.

d) Additional Modifications

Patent Owner argues that "the prior art would have taught the POSA to make asparagine substitutions, not glycine substitutions, to address alkaline stability concerns." PO Resp. 44. In other words, Patent Owner argues the prior art would have suggested making additional substitutions most notably in the asparagine residues of domain C. *Id.* at 45.

3. Petitioner's Reply

In response, Petitioner argues that

Abrahmsén and *Hober* each expressly pointed to a C(G29A) mutation (Ex. 1005, 2:32-37; Ex. 1006, 12), which was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence (*see, e.g.*, Ex. 1004, [2]). As the Board recognized, "*Abrahmsén* provides motivation for making [the G29A] mutation in **any of the IgG binding domains.**" (Decision, 24; *see also* Ex. 1057, 97:3-16 (Dr. Bracewell admitting that *Abrahmsén* discloses a G29A mutation to any of the five domains, including Domain C).)

Reply 2.

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A POSA would have reasonably expected success in combining these teachings to achieve the claimed affinity chromatography matrix given the well-known fact that each individual domain, including Domain C, has affinity for antibodies (Ex. 1004, [1]), as well as *Abrahmsén*'s confirmation that G29A “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1057, 99:13-101:21 Ex. 1005, 5:13-16; Ex. 1002 ¶131).

Id. at 3.

Petitioner argues that Patent Owner “has not disputed that *Abrahmsén* disclosed that G29A ‘would not interfere with folding to protein A or binding to IgG.’ (Ex. 1005, 2:32–37, 5:4–16; Ex. 1057, 109:20–110:17.) Nor does it take issue with its own statements in *Hober* that G29A is advantageous for ‘structural stability reasons.’ (Ex. 1006, 12.)” *Id.* at 9. Petitioner contends that Patent Owner’s lack of binding argument is contradicted by “*Abrahmsén* and *Hober*, which make clear that G29A does not affect the ability of an SPA ligand to bind to an antibody. (Ex. 1005, 2:32–37, 5:4–16; Ex. 1006, 12.)” *Id.* at 10.

Petitioner argues that “a POSA would have started with any one of the naturally occurring domains. (Decision, 26-28.) To then increase alkali stability, a POSA would have made the simplest, well-known substitution: G29A. (Section II.A.1–2; Ex. 1061 ¶¶8–15.)” Reply 11.

Petitioner argues “that Fab-binding was an inherent feature of a C(G29A)-based ligand—which [Patent Owner] does not appear to dispute. (Decision, 34; [Prelim.] Resp., 53–54.) In fact, [Patent Owner] acknowledges that ‘C(G29A)-based SPA ligands retained substantial Fab-binding ability.’ (Resp., 56–57.)” *Id.* at 14.

Petitioner argues that “Fab-binding is not being used [in the Petition] as part of a finding of a motivation to combine; rather, it is an inherent

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property [of the composition] being claimed. And necessarily present properties do not add patentable weight when they are claimed as limitations. *In re Kubin*, 561 F.3d 1351, 1357 (Fed. Cir. 2009).” Reply 15–16. Petitioner further argues that Patent Owner’s reliance on *Honeywell* is misplaced because “*Honeywell* had to do with an inherent property being used as a teaching in an obviousness analysis; it did not involve a limitation in the challenged claim reciting an inherent property.” Reply 15 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. De C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017); *see also Pernix Ireland Pain v. Alvogen Malta Operations*, 323 F. Supp. 3d 566, 607(D. Del. 2018)).

4. Patent Owner’s Sur-reply

Patent Owner argues that “Petitioners, and the Institution Decision, overlook an important point of consensus between the parties’ experts: the field of protein engineering is notoriously *unpredictable*.” Sur-reply 2. Patent Owner maintains that Petitioner has not identified a motivation to start from domain C. *Id.* at 3. Patent Owner argues that “Petitioners would have the Board look past the multitude of references teaching a preference for Domains B and Z—including Petitioners’ foundational references—and seize upon fleeting mentions of Domain C.” *Id.* at 7 (citing *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965)”). Patent Owner argues that “[m]ere sequence homology does not make the field predictable, as both experts observe, Ex. 2015 at 51:15-52:1, 56:4-12, 75:12-22, 73:16-20; Ex. 2025 ¶¶ 50-52; Ex. 2049 at 72:1-73:12, and as the vastly different Fab-binding properties of the near-identical Domains B and Z well illustrate, Ex. 2029 at 8.” *Id.* at 8–9.

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Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7–10.” Sur-reply 11.

5. *Analysis*

a) *Independent Claims 1 and 14*

Claims 1 and 14 of the ’142 patent are directed to a method of isolating a target compound using a chromatography matrix composition. The claims recite three active steps: (1) contacting, (2) adsorbing, and (3) eluting the target compound from the chromatography matrix. The claims further stipulate that the chromatography matrix composition (a solid support) has the following features: the ligand attached is attached to the matrix and the ligand is made up of at least two polypeptides comprising 55 contiguous amino acids of SEQ ID NO: 1⁶ having a G29A mutation. Claim 14 is similar to claim 1, except that claim 14 recites “at least 55 amino acids in alignment with SEQ ID NO. 1” instead of “at least 55 contiguous amino acids of modified SEQ ID NO. 1” as recited in claim 1.

(a) *Process Steps*

Petitioner asserts that the combination of Linhult, Abrahmsén, and Hober teaches or suggests the standard affinity chromatography process steps of (1) contacting, (2) adsorbing, and (3) eluting for the reasons set forth in the Petition. Pet. 16–31; Ex. 1002 ¶ 24.

⁶ Wild type amino acid sequence of domain C from *Staphylococcus* protein A (SPA). See Ex. 1001, 4:27, 6:35–36, 6:51–52.

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Linhult teaches making affinity chromatography columns with protein Z, Z(F30A), and other mutated variants. These modified proteins were covalently attached to HiTrap columns in Linhult using NHS-chemistry. Ex. 1004, 4. Linhult uses an affinity matrix column to isolate IgG and measures the loading capacity of the column after repeated cleaning in place (CIP) cycles. Ex. 1004, 4. In Linhult's studies, human polyclonal IgG was prepared and injected onto the columns in excess and "[a] standard affinity chromatography protocol was followed." *Id.* at 4. We find that Linhult's loading of IgG onto the column satisfies the contacting step as recited in the claims. Ex. 1004, 4, *see also id.* ("The columns were pulsed with TST (25mMTris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20) and 0.2 M HAc, pH 3.1. Human polyclonal IgG in TST was prepared and injected onto the columns in excess. A standard affinity chromatography protocol was followed for 16 cycles."). Linhult teaches that "the amount of eluted IgG was measured after each cycle to determine the total capacity of the column." *Id.* Linhult thereby expressly teaches the claimed contacting and eluting steps, and following standard chromatography protocols, the adsorbing step is implied. *Id.*; Ex. 1002 ¶ 24 ("After loading is completed, an additional step to wash out certain remaining impurities is employed. ([Ex. 1006 at 15–17]). Following the loading and wash steps, a different solution, typically one of low pH, is applied onto the column to elute the antibody"). Linhult also teaches a cleaning in place step using an alkaline cleaning agent. "The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min, resulting in a total exposure time of 7.5 h for Z(F30A) and mutants thereof." Ex. 1004, 4.

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Abrahmsén teaches using an IgG bound column for purifying the dimeric Z fragment from a supernatant. Ex. 1005, 9:60–10:15; Ex. 1002

¶¶ 64–68. Abrahmsén affinity purification protocol is as follows:

The supernatant was passed through the column at a speed of 12 ml/h and the amount of IgG binding material [i.e. the Z fragment] was analyzed before and after it was run through the column. The bound material was washed with TS [(150 mM NaCl 50 mM tris HC pH 7.5)] supplemented with 0.05% Triton X-100 and then TS and finally with 0.05 M ammonium acetate before elution with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.

Ex. 1005, 10:8–16. The contacting step in Abrahmsén’s protocol occurs when the “supernatant was passed through the column,” the adsorbing step occurs before or during the time “[t]he bound material was washed,” and the “elution” step occurs when the column is treated “with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.” *Id.* Although Abrahmsén column has IgG bound to the column, instead of an SPA domain, the reference still teaches the common chromatography steps of (1) contacting, (2) adsorbing, and (3) eluting a target molecule.

Hober teaches that “protein monomers can be combined-into multimeric proteins, such as dimers, trimers, tetramers, pentamers etc.”

Ex. 1006, 11. These monomer units can be linked with stretches of amino acids ranging from 0–15 amino acids. *Id.* Hober teaches

a matrix for affinity separation, which matrix comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which protein at least one asparagine residue has been mutated to an amino acid other than glutamine. . . The mutated protein ligand is preferably an Fc fragment-binding protein, and can be used for selective binding of IgG. . . the ligands present on the solid support comprising a multimer.

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Ex. 1006, 13. Hober describes a typical chromatographic run cycle consisting of: sample application of 10 mg polyclonal human IgG; extensive washing-out of unbound proteins; elution at 1,0 ml/min with elution buffer; followed by Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0,5 M NaOH of 1 hour. *Id.* at 37.

Patent Owner does not dispute that the references disclose the recited chromatography process of contacting, adsorbing, and elution as we have just described here. *See generally* PO Resp., *see id.* at 7 (citing Ex. 1002 ¶ 24; Ex. 2025 ¶ 43).

(b) Chromatography Matrix

The dispute between the parties is whether a person of ordinary skill in the art would have modified the process disclosed in Linhult using the G29A modified SPA C domain as disclosed in Abrahmsén. Pet. 22 (“Abrahmsén unequivocally discloses performing a G29A mutation on SPA’s C domain.”); *See* PO Resp. 17–54.

Linhult discloses a process for isolating one or more target compound(s) using chromatography matrices (solid support) comprising SPA ligands. Pet. 16–32. Linhult explains that SPA is a cell surface protein expressed by *Staphylococcus aureus* and consists of five highly homologous domains (E, D, A, B, and C). Ex. 1004, 1. Each of “[t]he five SPA domains show[s] individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” *Id.* (bracketing in original). “Due to the high affinity and selectivity of SPA, it has a widespread use as an affinity ligand for capture and purification of antibodies.” Ex. 1004, Abst., *see also*

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id. at 1 (“SPA has a widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.”).

Linhult explains that, in column chromatography, sodium hydroxide (NaOH) is probably the most extensively used cleaning agent for removing contaminants such as nucleic acids, lipids, proteins, and microbes, and a CIP step is often integrated in the protein purification protocols using chromatography columns. Ex. 1004, 1. “Unfortunately, protein-based affinity media show high fragility in this extremely harsh environment, making them less attractive in industrial-scale protein purification. SPA, however, is considered relatively stable in alkaline conditions.” *Id.* at 2. Linhult explains that the combination of asparagine with a succeeding glycine is the most sensitive amino acid sequence to alkaline conditions. *Id.* Linhult teaches that “[a]n exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is [sensitive to alkaline conditions and is also] a cleavage site for hydroxylamine.” *Id.*

Petitioner’s expert, Dr. Cramer avers that the “Z” domain referenced in Linhult refers to a synthetic version of the wild-type (i.e., natural) B domain of SPA, in which the naturally occurring glycine in the Asn₂₈-Gly₂₉ dipeptide sequence is replaced by an alanine residue to create an Asn₂₈-Ala₂₉ dipeptide sequence. Ex. 1002 ¶ 30 (citing Ex. 1004, 2, Fig. 1(a); Ex. 1007, 3, Fig. 1); ¶ 31 (citing Ex. 1005). We credit Petitioner’s expert, Dr. Cramer for establishing that the C domain sequence disclosed in Linhult contains 55 amino acids in SEQ ID NO: 1 as claimed. Ex. 1002 ¶ 106 (showing a sequence alignment), *see also id.* ¶ 292 (showing sequence alignment of domain C of Abrahmsén with SEQ ID NO: 1).

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According to Abrahmsén, the IgG binding domains of E, D, A, B, and C domains of SPA were known. *See* Ex. 1005, 3:25–35, 4:34–37, Fig. 2. Relying on “computer analysis [Abrahmsén] surprisingly showed that the Gly in the Asn-Gly dipeptide sequence could be changed to an Ala. This change was not obvious as glycines are among the most conserved amino acids between homologous protein sequences due to their special features.” *Id.* at 5:7–9. Abrahmsén teaches that, in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. Thus, Abrahmsén provides motivation for making this mutation in any of the IgG binding domains of E, D, A, B, and C domains of Staphylococcal protein A. Abrahmsén teaches recombinant DNA fragments coding for any of the E, D, A, B, and C domains of Staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon. *Id.* at 2:33–37.

Abrahmsén, like Linhult, exemplifies the cloning of and expression of the Z-fragment. Ex. 1005, 7:65–10:56. Abrahmsén teaches that “the Z-region is the part of the Z-fragment coding for the IgG binding domain.” *Id.* at 3:39–41. Abrahmsén purifies the recombinant Z protein using an IgG column. *Id.* at 10:26–28. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén, therefore, reasonably suggests making multimeric constructs. Abrahmsén also uses column chromatography to purify a Z domain containing protein.

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Hober teaches a multimer ligand that

also comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A. In this embodiment, it is preferred that asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids. In an embodiment advantageous for structural stability reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to, an alanine residue. Also, it is advantageous for the structural stability to avoid mutation of the asparagine residue in position 52, since it has been found to contribute to the α -helical secondary structure content of the protein A molecule.

Ex. 1006, 12, *see also id.* at 9 (“SEQ ID NO 1 defines the amino acid sequence of the B-domain of SpA”).

Here, the teachings of Linhult, Abrahmsén, and Hober suggest mutating the glycine at position 29 for an alanine in any one of the IgG binding domains of E, D, A, B, or C of SPA in order to avoid protein degradation. Ex. 1004, 2; Ex. 1005, 5:4–9. We, therefore, agree with Petitioner that the art expressly suggests that the glycine codon can be mutated for an alanine codon in any one of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111). Attaching any one of SPA mutated IgG binding domains E D A B or C to a matrix using “known ligand-construction methods to yield a predictable result[] (e.g., the claimed affinity chromatography matrix)” would have been obvious. Pet. 25 (citing Ex. 1002 ¶ 130). As the Federal Circuit has explained, “[w]here a skilled artisan merely pursues ‘known options’ from ‘a finite number of identified, predictable solutions,’ the resulting invention is obvious under Section 103.” *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421).

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Accordingly, we agree with Petitioner that the combination of Linhult Abrahmsén, and Hober expressly suggests mutating the glycine codon for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111). We also agree that the cited art teaches using these mutant SPA domains in column chromatography for the isolation of antibodies.

We address Patent Owner’s contentions below.

(2) *Response*

(a) *Matrix*

We do not find Patent Owner’s argument that the Petition fails to identify a reason to select domain C persuasive. PO Resp. 17–38. Specifically, we are not persuaded by Patent Owner’s contention that just because nobody was working on domain C at the time the invention was filed, one of skill in the art would not have been motivated to select domain C. *See* PO Resp. 31 (“A general recognition that there exist five naturally occurring protein A domains is not a motivation to use each of them as a starting point for the claimed mutations”).

Petitioner’s articulated obviousness ground is premised on the knowledge that *any one* of the five SPA IgG binding domains are known to bind IgG and can function as a ligand for the purification of antibodies. Linhult and Abrahmsén both expressly suggest that the glycine codon at position 29 can be mutated for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Ex. 1004, 2; Ex. 1005, 2:32–37. Here, the SPA IgG binding domains comprise a short list of 5 members: E, D, A, B, or C. Of these 5 members, the glycine at position 29 in domain B has already been mutated to an alanine to create a domain Z which has been shown to

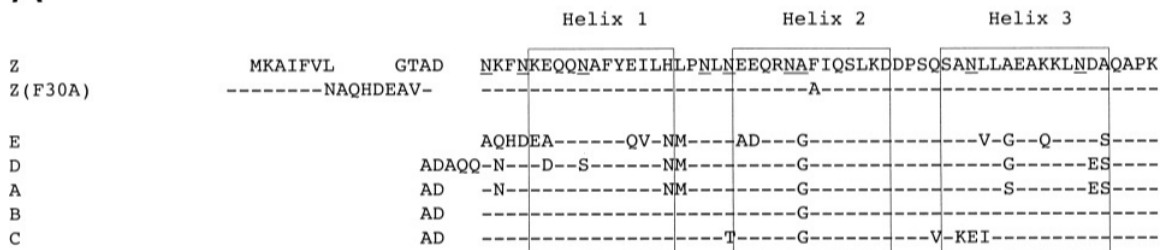
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retain IgG binding activity. Ex. 1004, 6 (Fig. 3). Figure 1A of Linhult is reproduced below.

A



Linhult's Figure 1A, reproduced above, shows the amino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C). The three boxes show the α -helices. Ex. 1004, 2; Ex. 1005, Fig. 2.

Here, Linhult and Abrahmsén show that the IgG binding domains of SPA – E, D, A, B, and C share many structural similarities. *See* Ex. 1004, 2 (Fig. 1(a) (reproduced above)); Ex. 1005, 3:25–35. As discussed in our Institution Decision (Dec. 30–31), there are a finite number—five (5)—SPA IgG binding domains and each possesses the dipeptide sequence Asp-Gly known to be a target for alkaline protein degradation. Therefore, the solution of mutating the glycine at position 29 for an alanine to remove the alkaline sensitive sequence is not a product of innovation, but of ordinary skill and common sense. *See Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421). It is well established that

[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate

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making them to try to obtain compounds with improved properties.

In re Deuel, 51 F.3d 1552, 1558 (Fed. Cir. 1995).

There is also an express teaching in both Linhult and Abrahmsén to mutate the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which further supports why one of skill in the art would have reason to incorporate the mutation into any one of the IgG binding domains that has the Asn-Gly dipeptide. *See, e.g., SIBIA Neurosciences, Inc. v. Cadus Pharm. Corp.*, 225 F.3d 1349, 1358–59 (Fed. Cir. 2000) (stating that an express teaching in the prior art suggesting a particular modification establishes obviousness).

Because the G29A modification would have provided ligands that are less susceptible to alkaline conditions and are resistant to hydroxylamine cleavage, Petitioner has provided a sufficient evidence-backed reason for making the modification in any one of the domains. Pet. 16–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

(b) Reasonable Expectation of Success

We are not persuaded by Patent Owner’s contention that there is no reasonable expectation of success in using a G29A mutation in domain C. PO Resp. 48–54.

Linhult explains that removing the Asp–Gly amino acid combination not only results in the removal of the hydroxylamine cleavage site, but also creates a product that is more alkaline resistant. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for

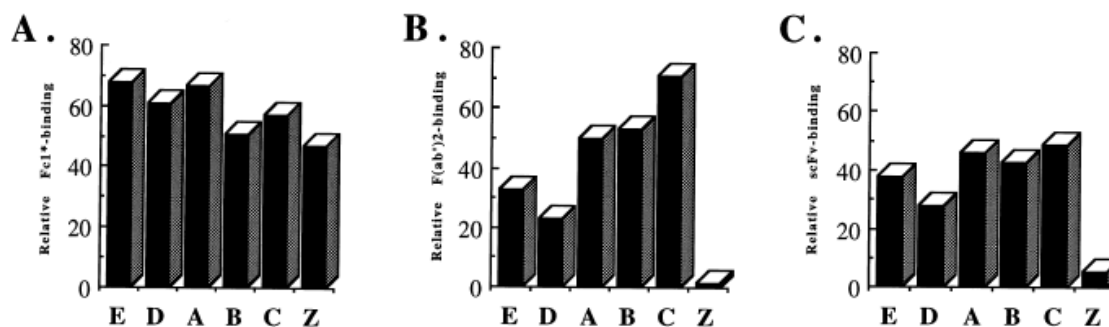
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hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”).

Abrahmsén teaches that this Asn-Gly amino acid combination is present in all five SPA IgG binding domains and that mutating the dipeptide would not interfere with IgG binding. Ex. 1005, 4:56–58 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine. [T]his sequence is kept intact in all five IgG binding domains of protein A. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”). Abrahmsén’s conclusion that the mutation would not interfere with binding to IgG is supported by Abrahmsén (*see* Ex. 1005, 9:60–10:35), Linhult (*see* Ex. 1004, 6 (Fig. 3)), and Jansson (Ex. 2029).⁷ Jansson’s Fig. 3, reproduced below:



Jansson Figure 3 (Panel A), reproduced above, shows a side-by-side comparison of Fc1*, Fab, and scFv binding to SPA domains. The figure shows that the single G29A mutation between domain B and domain Z

⁷ Patent Owner cites Jansson (Ex. 2029) for the position that domain Z has negligible binding to Fab. *See* PO Resp. 40–41. However, claims 1 and 14 are not limited to Fab binding. Indeed, the claims do not even require IgG binding.

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results in a protein that is able to bind IgG.⁸ Comparing panel A - domain B domain with panel A - domain Z, the relative binding remains close to 50%, indicating that the G29A mutation between domains B and C does not interfere with IgG binding. Ex. 2029, 6. This result is predicted by Abrahmsén's computer modeling and substantiated by Abrahmsén domain Z purification and Linhult's IgG purification. *See* Ex. 1005, 4:56–58, 9:60–10:35; Ex. 1004, 6 (Fig. 3).

“Obviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success.” *In re Droge*, 695 F.3d 1334, 1338 (Fed. Cir. 2012) (quoting *In re Kubin*, 561 F.3d 1351, 1360 (Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894, 903–04 (Fed.Cir.1988)); *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016) (explaining that the expectation of success issue involves a showing of “a reasonable expectation of achieving *what is claimed*”) (emphasis added). “Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention.” *Pharma Stem Therpeutic, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1363–1364 (2007).

Here, the record supports that each individual SPA domain, including the C domain, has affinity for IgG antibodies. Ex. 1004, 1 (“The five SPA

⁸ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the “IgG control” in Jansson. Patent Owner's counsel explains that “Part A is Fc binding. So that is, I believe the way they did this experiment was with Fc fragments, but it's generally acknowledged, you know, these antibodies all have an Fc domain if they're a whole antibody and that reflects the fact that all of these domains A, B, C, D and E and domain Z, which is B with the G29A mutation, retain this Fc binding.” Tr. 70:6–11.

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domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” (bracketing in original)). Abrahmsén suggests making a mutation of Asn-Gly coding constellation in *any one* of the SPA domains by replacing a glycine codon with an alanine codon to remove the Asn-Gly dipeptide sequence known to be sensitive to hydroxylamine degradation. *See* Ex. 1005, 4:56–5:16, *see also id.* Fig. 2 (showing the Asn-Gly coding constellation in all SPA domains); Ex. 1006, 2 (“the shortest deamidation half times have been associated with the sequences –asparagine–glycine and – asparagine–serine”). Abrahmsén’s confirms that a G29A mutation on SPA would not interfere with folding of SPA protein and binding to antibodies. Ex. 1005, 5:13–16 (“by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”), 9:60–10:35 (using IgG columns to purify protein Z dimers). Abrahmsén’s computer modeling suggests that IgG binding is not impacted by the mutation, and this is confirmed by Linhult’s experiments showing that the G29A mutant of domain B (a.k.a. domain Z) binds IgG. Ex. 1004, 6 (Fig. 3); *see also* Ex. 1005, 9:60–10:35.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

We are not persuaded by Patent Owner’s contention that the information gained by computer modeling of the SPA native domain B-IgG

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crystal structure could not be extrapolated to other SPA domains that are structurally very similar.

As Petitioner's expert, Dr. Cramer, explains

[i]t was well known that the researchers who developed the Z domain based on the wild-type B domain (rather than any of the other four SPA domains) did so for two reasons. (*See, e.g.*, Ex. 1007 at 109.) First, a crystal structure of the wild-type B domain binding to an antibody happened to be available in 1981 for analysis. (*See, e.g., id.*; Ex. 1005 at col. 4:56-68; Ex. 1017.) And, second, *their work would be informative of mutations that could be done on all five of the highly homologous SPA domains more generally.* (*See, e.g.*, Ex. 1005 at col. 2:32-37; Ex. 1007 at 109; Ex. 1008 at 639, Fig. 1.)

Ex. 1002 ¶ 33 (emphasis added). Dr. Cramer further explains that “[t]hey did the computer modeling based on that complex because that’s the crystal structure that they had. It wasn’t done because the B domain is special. . . . And then there’s several other places where they state clearly that they could also do the other domains with expected similar results.” Ex. 2015, 138:8–22.

We credit Dr. Cramer’s testimony here and agree with Petitioner that, when taken together, the teachings of Linhult, Abrahmsén, and Hober provide a reasonable expectation of success at arriving at a chromatography composition that contains the SPA domain C ligand with the G29A mutation that can be used in a process for purifying IgG. Pet. 24 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 124–126), *see id.* at 48–49 (citing Ex. 1006, 10–12; Ex. 1002 236–263).

(c) No Teaching Away

We are also not persuaded by Patent Owner’s contention that the art teaches away from the G29A substitution because it interferes with Fab

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binding. *See* PO Resp. 41 (citing Ex. 2009 at 2; Ex. 2010 at 2; Ex. 2012 at 25–26; Ex. 2029 at 7); Sur-reply 9.

Neither claim 1 nor claim 14 recite a need to bind the Fab region of an antibody or that the target molecule is Fab. All that is required by these claims is that they adsorb a target molecule and that you can elute the target molecule from the matrix. The target molecule, therefore, can reasonably encompass IgG.

Petitioner’s articulated rationale is that there was an expectation that the composition binds antibodies, including monoclonal antibodies, and therefore, would be useful in a process of for isolating antibodies. Petitioner contends that:

A POSA would have also reasonably expected such a combination to achieve a process for isolating one or more target compounds using the recited affinity chromatography matrix given the well-known fact that each individual SPA domain, including the C domain, has affinity for antibodies (Ex. 1004, [1]) as well as *Abrahmsén*’s confirmation that a G29A mutation on SPA “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1005, 5:13-16). (Ex. 1002 ¶131.)

Pet. 25; Reply 8 (“A POSA would not have been motivated only by Fab-binding ability, as even Dr. Bracewell agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’ (Ex. 1057, 75:17-76:4, 113:23-114:11, 157:24-158:9; Ex. 1061 ¶29)”).

The law does not require that the teachings of the reference be combined for the reason or advantage contemplated by the inventor, as long as some suggestion to combine the elements is provided by the prior art as a whole. *In re Beattie*, 974 F.2d 1309, 1312 (Fed. Cir. 1992); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976); *see In re Kemps*, 97 F.3d 1427, 1430

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(Fed. Cir. 1996) (“[T]he motivation in the prior art to combine the references does not have to be identical to that of the applicant to establish obviousness.”).

Here, Linhult teaches that “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” Ex. 1004, 1 (bracketing in original) (citation omitted). Linhult, therefore, teaches that *any one* of the SPA IgG binding domains E, D, A, B, or C can bind the Fc region of an antibody and can therefore be used as a ligand for purifying IgG antibodies. In addition, the combination of Linhult and Abrahmsén suggests making the G29A mutation in each of the domains because it would provide ligands that are less susceptible to protein degradation. Ex. 1004, 2; *see also* Ex. 1005, 2:33-37 (“[A] recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.”).

Patent Owner contends that the G29A mutation would lead to a reduction in the Fab binding of domain C, and therefore, would lead away from making the mutation. PO Resp. 40–41 (citing Ex. 2010, 2; Ex. 2011, 25; Ex. 2012, 25–26; Ex. 2013, 2–3; Ex. 2025 ¶¶ 105–109; Ex. 2029, 6–7). Patent Owner’s cited references are directed to Fab binding. But claims 1 and 14 are not limited to Fab binding. Showing that the G29A mutation interferes with Fab binding does not discredit the ability of a mutated SPA domains E, D, A, B, or C to bind the Fc portion of IgG. *See, e.g.*, Ex. 2013, 3 (“The site responsible for Fab binding is structurally separate from the

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domain surface that mediates Fcγ⁹ binding.”). Accordingly, we are not persuaded by Patent Owner’s contention that with respect to claims 1 and 14 that the art teaches away from Petitioner’s proposed combination.

(d) Additional Modifications

We also disagree with Patent Owner’s contention that the ordinary artisan would not stop with a single G29A mutation in a SPA domain. *See* PO Resp. 44–48. Here, Abrahmsén expressly suggests making only a single mutation. Specifically, Abrahmsén contemplates “a recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon” without additional mutations. Ex. 1005, 2:33–37.

(3) Summary

We find that Petitioner has shown by a preponderance of the evidence that the combined teachings of at least Linhult and Abrahmsén suggests the use of *any one* of the SPA IgG binding domains E, D, A, B, or C as the starting ligand for purifying IgG antibodies, and that making the G29A mutation in *any one* of the domains would have been obvious because it would have provided ligands that are less susceptible to alkaline conditions

⁹ Fcγ is the constant region of IgG involved in effector function. Ex. 2013, 1. Specifically, “[t]he Fcγ binding site has been localized to the elbow region at the CH2 and CH3 interface of most IgG subclasses, and this binding property has been extensively used for the labeling and purification of antibodies.” *Id.* In other words, Fcγ and Fc terminology are used interchangeably in the art.

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and are resistant to hydroxylamine cleavage. Pet. 16–25; Reply 3–5;

Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

Having considered the evidence and argument of record, which we have described above and find persuasive, we determine that Petitioner has shown by a preponderance of evidence of record that the combination of Linhult, Abrahmsén, and Hober teaches each of the limitations of claims 1 and 14. Petitioner not only has articulated a sufficient motivation for making the combination but has also established that there is a reasonable expectation of success for the binding of an IgG antibody to a SPA domain that contains an G29A mutation.

b) Claims 4 and 17

Petitioner argues that “[t]he ‘capab[ility] of binding to the Fab part of an antibody,’ as recited in claims 4 and 17, is an inherent property of the claimed C(G29A)-based SPA ligand.” Pet. 28 (citing Ex. 1002 ¶¶ 141–148). Petitioner contends that a person of ordinary skill in the art did not need to recognize the Fab binding property of domain C to be motivated to select that domain for modification. “A POSA would not have been motivated only by Fab-binding ability,[] as even Dr. Bracewell [Patent Owner’s expert] agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’” Reply 8 (citing Ex. 1057, 75:17–76:4, 113:23–114:11, 157:24–158:9; Ex. 1061 ¶ 29).

Patent Owner argues that

[t]he very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding “negligible” when implemented in Domain B. Ex. 2009 at 2; *see also, e.g.*, Ex. 2010 at 2 (“Fab binding activity is located to a region determined by

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helices 2-3, including the position mutated to yield the Z domain.”); Ex. 2011 at 25 (“[I]t only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine to an alanine”); Ex. 2012 at 25-26 (“[D]omain Z containing a single G29A-substitution compared to domain B exhibits little or no [Fab] binding. This might be due to the substitution since the C_β of the alanine would perturb the interaction between the two molecules.”).

PO Resp. 41.

Because claim 4¹⁰ is directed to a “[a] process for isolating one or more target compound(s)” and identifies that the target compound is “the Fab part of an antibody” (Ex. 1001, 15:63–64) the prior art needs show a reasonable expectation that a mutated SPA ligand binds Fab. Without such a showing, there is no reasonable expectation that the process would result in the purification of a Fab target. In other words, because the claims are process claims, Petitioner needs to establish that a mutated SPA domain would reasonably bind a Fab fragment.

We agree with Petitioner, that Linhult establishes that a G29A mutation in domain B (resulting in domain Z) does not interfere with IgG binding. *See* Ex. 1004, 6 (Fig. 3 showing IgG binding with domain Z). Linhult, however, is silent with respect to domain Z’s ability to bind to Fab fragments. *See generally* Ex. 1004. Abrahmsén similarly establishes domain Z binding to IgG but is also silent with respect to domain Z binding Fab. *See generally* Ex. 1005. Hober also does not disclose Fab binding of a mutant SPA domain. *See generally* Ex. 1006. Thus, each of Linhult,

¹⁰ Claim 14 and 17 recite similar limitations.

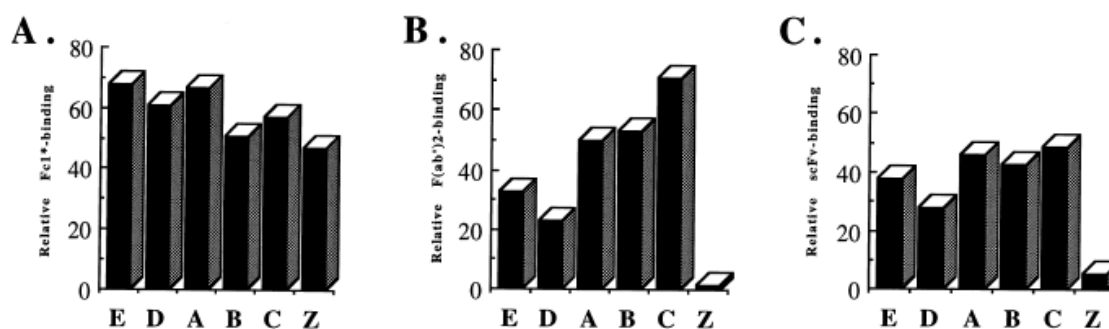
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Abrahmsén, or Hober is silent with respect to Fab binding to a mutated SPA domain.

Jansson (Ex. 2029), cited by Patent Owner, supports the position that Fab binding to mutated SPA domains is unpredictable. Jansson, just like Linhult, recognizes that “[a]ll [SPA] domains bound to a recombinant human IgG1 Fc fragment with similar strength. For the first time, binding to human Fab was demonstrated for all *native SPA domains*, using both polyclonal F(ab')₂ and a recombinant scFv fragment as reagents.” Ex. 2029, Abstract (emphasis added). Jansson, however, establishes that “the engineered Z domain showed a considerably lower affinity for Fab as compared to the native domains.” *Id.* Jansson Fig. 3, reproduced below, shows that the G29A mutation results in a loss of Fab binding ability.



Jansson Figure 3, reproduced above, shows the side-by-side comparison of Fc1*¹¹, Fab, and scFv binding and confirms what was already suggested in Linhult, Abrahmsén, and Hober – that a composition containing the G29A mutation in a SPA domain can bind IgG. Ex. 2029, 6 (Fig. 3 (*compare* Panel A- domain B, *with* Panel A- domain Z)). Panel B in Jansson Figure 3, however, shows that the single G29A mutation between domain B and

¹¹ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the IgG control in Jansson. Fc1* is functionally equivalent to Fcγ in SPA binding.

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domain Z results in the loss of Fab binding. Ex. 2029, 6 (Fig. 3 (*compare* Panel B- lane B, *with* Panel B- lane Z)). At the time the invention was made it was also known that “[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding.” Ex. 2013, 3. Thus, on this record, establishing that a mutation that does not interfere with IgG binding does not inform one of skill in the art about the ability of a mutated SPA domain to bind Fab.

We, therefore, agree with Patent Owner’s contention that based on the prior art, the Fab binding capacity was unknown with the modification as suggest by the combination of Linhult, Abrahmsén, and Hober.¹² Patent Owner has provided evidence that Fab binding capacity of a mutated SPA domain protein is unpredictable. *See* PO Resp. 56–57 (Ex. 2029, 5–6). The disclosures in the prior art, therefore, support Patent Owner’s position that “the SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” PO Resp. 54–55 (citing Ex. 2025 ¶ 123; Ex. 2030, 18–19).

Because the art does not support the conclusion that G29A mutation in a SPA domain ligand binds Fab, Petitioner has not established by a

¹² In *JSR Corporation et al. v. Cytiva Bioprocess R&D AB et al.*, IPR2022-00036, Paper 41 at 44–49 (PTAB April 19) (Final Written Decision) we determined that the capability of SEQ ID NO:1 to bind Fab is an inherent feature of the structure claimed. The present claims, however, are directed to a method of isolating Fab which requires prior knowledge that the ligand binds Fab. Fab is a digestion product of a whole IgG molecule treated with papain and is not naturally found in IgG samples. *See above* I.F. In other words, when isolating IgG with a column containing SEQ ID NO: 1, there would be no elution of Fab because the fragments are not present in an IgG containing sample.

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preponderance of the evidence of record that the process of isolating Fab target using a mutated SPA domain C ligand would have been obvious based on the combined teachings of Linhult, Abrahmsén, and Hober.

c) Claims 3, 16, and 28–30

Claims 3, 27, and 28 depend either directly or indirectly from claim 1, and claims 16, 29, and 30 depend either directly or indirectly from claim 14. Claim 3 and 16 recite the additional limitation “wherein the chromatography matrix has retained at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.” Ex. 1001, 15:52–62. Claim 27 and 29 recite “comprising a step of exposing the chromatography matrix to 0.1 to 0.5 M NaOH.” Claim 29 and 30 recite “repeated exposure of the chromatography matrix to the NaOH for at least 80 cycles.”

We are not persuaded by Patent Owner’s argument that “none of Petitioners’ cited references actually describe a C(G29A)-based SPA ligand, let alone provide alkaline stability data or test results concerning the same, the POSA is simply left to guess at how such a ligand would perform.” PO Resp. 50 (citing Ex. 2025 ¶¶ 121–124).

For the reasons discussed above (II.E.5.2.a), we find that Petitioner has shown by a preponderance of evidence that there is a reason to make the G29A mutation in any one of the SPA IgG binding domains, including domain C, and that the use of the mutated protein would reasonably result in a matrix that can be used to purify IgG. Linhult teaches a CIP protocol with at 0.1 to 0.5M NaOH was a well-known and conventional technique. Pet. 30 (citing Ex. 1004, 1–2, 4–5; Ex. 1002 ¶ 152); Ex. 1004, 6 (“Figure 3, the Z(N23T) mutant shows higher resistance to alkaline conditions than the Z domain when exposed to high pH values.”); *see also* Ex. 1006, 39

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(“Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0,5 M NaOH of 1 hour”). “Between each cycle [in Linhult], a CIP-step was integrated. The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min.” Ex. 1004, 4. “After 16 [CIP] cycles, giving a total exposure time of 7.5 h, the column with the Z(F30A)-matrix shows a 70% decrease in capacity.” Ex. 1004, 5; *see also* Ex. 1006, 39 (“Each cycle [in Hober] was repeated 21 times resulting in a total exposure time between the matrix and the sodium hydroxide of 20 hours for each different matrix”). Both Linhult and Hober recognize that repeated exposure of a SPA chromatography ligand leads to a reduction of the binding capacity over time. That Linhult recognizes that additional mutations could further improve alkaline stability does not detract from Linhult’s teaching that a composition containing the single G29A mutation in SPA domain B retains IgG binding. Ex. 1004, 6, *see id.* at 4 (“The Z-domain already possesses a significant tolerance to alkaline conditions.”).

Petitioner has shown by a preponderance of the evidence of record that there is a reason for making the G29A mutation in *any one* of the four remaining SPA domains in order to produce a SPA product that is more alkaline stable and would reasonably bind IgG. *See* Pet. 26–28, 30; Reply 20–21; *see* Ex. 1002 ¶¶ 115–119.

d) Claims 2, 12, 15, and 25

Claims 2 and 12 depend from claim 1, and claims 15 and 25 depend from claim 14. Claim 2 and 15 recite the additional limitation that “ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.” Claims 12 and 25 recite the additional element that “the ligand comprises an amino acid sequence that comprises 2-8 of the polypeptides.”

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Petitioner asserts that Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims. *See* Pet. 25–28, 30.

Patent Owner does not offer arguments addressing Petitioner’s substantive showing with respect to the additional elements added by dependent claims 2, 12, 15, and 25. *See generally* PO Resp.

We have reviewed Petitioner’s arguments and the underlying evidence cited in support and determine that Petitioner establishes that of Linhult, Abrahmsén, and Hober teaches the additional limitations of these dependent claims. Pet. 26 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 112–114), *see id.* at 30 (citing Ex. 1002 ¶¶ 149–150); Ex. 1004, 4 (“a multimerization of the domain to achieve a protein A–like molecule”); Ex. 1005, 9:15–10:35. Hober also teaches that monomeric mutant proteins can be combined into multimeric proteins, such as dimers, trimers, tetramers, pentamers, and other multimers. Ex. 1006, 11. Hober also discloses that the multimer comprises mutant monomer units “linked by a stretch of amino acids preferably ranging from 0 to 15 amino acids, such as 5-10.” *Id.*

The preponderance of evidence of record supports Petitioner’s contentions with respect to claims 2, 12, 15, and 25.

6. Summary

For the foregoing reasons, we determine that Petitioner has shown by a preponderance of evidence that of claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the ’142 patent are unpatentable based on the combination of Linhult, Abrahmsén, and Hober.

For the reasons discussed above, Petitioner has not shown by a preponderance of evidence that of claims 4 and 17 of the ’142 patent are unpatentable.

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F. *Other Asserted Grounds*

a) *The '041 IPR Grounds Based on Other Various Combinations of Linhult, Abrahmsén, and Hober*

Petitioner also asserts that claims 1–4, 12, 14–17, and 25 are unpatentable as obvious over Linhult and Abrahmsén (Pet. 18–30); that claims 1–7, 10–20, and 23–26 are unpatentable as obvious over Linhult and Hober (*id.* at 30–48); and that claims 1–7, 10–20, 23–26 are unpatentable as obvious over Abrahmsén and Hober (*id.* at 49–60) under 35 U.S.C. §103(a).

Because Petitioner has already shown that the challenged claims 1–3, 5–7, 10–16, 18–20, and 23–30 are unpatentable over Linhult, Abrahmsén, and Hober as obvious, as discussed *supra*, we do not reach these claims in these additional asserted grounds as to those claims. *See Beloit Corp. v. Valmet Oy*, 742 F.2d 1421, 1423 (Fed. Cir. 1984) (“The Commission . . . is at perfect liberty to reach a ‘no violation’ determination on a single dispositive issue.”); *Boston Sci. Scimed, Inc. v. Cook Grp., Inc.*, 809 F. App’x 984, 990 (Fed. Cir. 2020) (recognizing that “[t]he Board has the discretion to decline to decide additional instituted grounds once the petitioner has prevailed on all its challenged claims”).

Petitioner has not shown that the challenged claims 4 and 17 are unpatentable over Linhult, Abrahmsén, and Hober. We note that each of Linhult, Abrahmsén, and Hober is silent with respect to Fab binding to a mutant SPA domain. *See above* II.E.5.b. Patent Owner asserts, and we agree, that there is no reasonable expectation that a G29A SPA domain mutant would bind Fab. Specifically, Patent Owner’s cited references show that a G29A mutation in domain B results in a domain Z matrix composition that *does not* bind Fab. *See* Ex. 2029, 6 (Fig. 3 (*compare* Panel B- domain B,

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with Panel B- domain Z)); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”). For the reason discussed above (II.E.5.b), Petitioner has not shown by a preponderance of the evidence of record that a G29A SPA domain mutant would bind a Fab fragment so that a process of using the G29A SPA domain ligand would reasonably result in the purification of the Fab target. Petitioner’s deficiency with respect to claims 4 and 17 persists whether the grounds are based on the combination of Linhult, Abrahmsen, and Hober or the related combinations Linhult and Abrahmsen; Linhult and Hober; or Abrahmsen combined with Hober.

b) The '044 IPR Grounds Based on Various Combinations of Berg¹³, Linhult, Abrahmsén, and Hober

Petitioner asserts that claims 1–7, 10–20, and 23–30 are unpatentable as obvious over Berg¹⁴ and Linhult ('044 IPR Pet. 18–30); that claims 2, 3, 15, and 16 are unpatentable as obvious over Berg, Linhult, and Hober (*id.* at 30–48); that claims 1, 2, 5–7, 10–15, 18–20, and 23–26 are unpatentable as obvious over Berg and Abrahmsén (*id.* at 49–60); and that claims 2–4, 15–17, and 27–30 are unpatentable as obvious over Berg, Abrahmsén, and Hober (*id.* at 49–60) under 35 U.S.C. §103(a).

¹³ Berg et al., US 2006/0134805 A1, published June 22, 2006. Ex. 1018.

¹⁴ We recognize that there is a dispute between the parties whether Berg qualifies as a 35 U.S.C. §102(a) date reference or a §102(b) date reference. *See* Pet; PO Resp., Reply, and Sur-reply. Because we do not need to reach these additional asserted grounds based on Berg beyond addressing whether Berg teaches Fab binding in the context of a mutant SPA domain ligand to establish that Berg does not address the deficiency of the Linhult, Abrahmsén, and Hober combination, we do not need to address the prior art status of Berg.

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Berg relates to a chromatography matrix to which antibody-binding protein ligands are immobilized. Ex. 1018, Abstract. Petitioner relies on single paragraph in Berg, paragraph 29, for teaching antibody binding ligands including SPA domain C. *See* '044 IPR Pet. 23, 45. The remainder of the Berg reference is directed to the structure of the chromatography matrix. *See generally* Ex. 1018. A review of Berg shows that SPA is mentioned at three locations in the reference. *See* Ex. 1018 ¶¶ 28, 29, and claim 12. Paragraph 29 of Berg suggests using ligands made up of one or more domains A, B, C, D, and E, and preferably domain B and/or domain C. Ex. 1018 ¶ 29. Just like Linhult, Abrahmsén, and Hober, Berg also does not say anything about the ability of a mutant SPA domain ligand, including a domain C ligand, to bind Fab.

Patent Owner, however, cites prior art references to establish that at the time the invention was made there was no expectation that a G29A SPA domain mutant would bind Fab. *See above* II.E.5.b; *see* '044 IPR PO Resp. 45, 55, 56, 60. Specifically, Patent Owner's cited references showing that a G29A mutation in domain B results in a domain Z matrix composition that does not bind Fab. *See id.*; Ex. 2029, 6 (Fig. 3 (compare Panel B- domain B, with Panel B- domain Z)); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”).

Berg, therefore, does not address Fab binding in the context of a mutant SPA domain ligand, specifically domain C, nor does Berg explain why one of ordinary skill in the art would have reasonably expected domain C to retain the ability to bind Fab when other SPA domain mutants do not retain this feature. Because Berg does not address the deficiency of

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Linhult, Abrahmsén, and Hober as identified by Patent Owner and discussed above (II.E.5.b), any combination of Berg in conjunction with Linhult, Abrahmsén, and/or Hober would not address the missing limitation of claims 4 and 17. Therefore, we do not reach these additional asserted grounds based on Berg beyond addressing whether Berg teaches this missing limitation. *See Beloit Corp.*, 742 F.2d at 1423.

III. CONCLUSION¹⁵

For the foregoing reasons, we determine that Petitioner has demonstrated by a preponderance of the evidence that claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the ’142 patent are unpatentable, and that claims 4 and 17 have not been shown unpatentable on the bases set forth in the following table.

¹⁵ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this decision, we draw Patent Owner’s attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. See 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. See 37 C.F.R. § 42.8(a)(3), (b)(2).

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In summary:

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–4, 12, 14–17, 25	103(a)	Linhult, Abrahmsén ¹⁶		4, 17
1–7, 10–20, 23–26	103(a)	Linhult, Hober ¹⁷		4, 17
1–7, 10–20, 23–30	103(a)	Linhult, Abrahmsén, Hober	1–3, 5–7, 10–16, 18–20, 23–30	4, 17
1–7, 10–20, 23–26	103(a)	Abrahmsén, Hober ¹⁸		4, 17
Overall Outcome			1–3, 5–7, 10–16, 18–20, 23–30	4, 17

¹⁶ As explained above (II.F.a), we do not reach claims 1–3, 12, 14, 15, and 25 in this '041 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

¹⁷ As explained above (II.F.a), we do not reach claims 1–3, 5–7, 10–16, 18–20, 23–26 in this '041 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

¹⁸ As explained above (II.F.a), we do not reach claims 1–3, 5–5, 10–16, 18–20, and 23–26 in this '041 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1-7, 10-20, 23-26	103(a)	Berg, Linhult ¹⁹		4, 17
2, 3, 15, 16	103(a)	Berg, Linhult, Hober ²⁰		
1, 2, 5-7, 10-15, 18-20, 23-26	103(a)	Berg, Abrahmsén ²¹		
2-4, 15- 17, 27- 30	103(a)	Berg, Abrahmsén, Hober ²²		4, 17
Overall Outcome			1-3, 5-7, 10-16, 18-20, 23-30	4, 17

¹⁹ As explained above (II.F.b), we do not reach claims 1-3, 5-7, 10-16, 18-20, and 23-26 in this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²⁰ As explained above (II.F.b), we do not reach this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²¹ As explained above (II.F.b), we do not reach this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²² As explained above (II.F.b), we do not reach claims 2, 3, 15, 16, and 27-30 in this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

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IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the preponderance of the evidence of record has shown that claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the '142 patent are found unpatentable;

ORDERED that the preponderance of the evidence of record has not shown that claims 4 and 17 of the '142 patent are found unpatentable; and

FURTHER ORDERED because this is a final written decision, the parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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Paper: 40
Date: May 18, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

JSR CORPORATION and JSR LIFE SCIENCES, LLC,
Petitioner,

v.

CYTIVA BIOPROCESS R&D AB,
Patent Owner.

IPR2022-00042
IPR2022-00045
Patent 10,875,007 B2

Before ULRIKE W. JENKS, SHERIDAN K. SNEDDEN, and
SUSAN L. C. MITCHELL, *Administrative Patent Judges*.

SNEDDEN, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining Some Challenged Claims Unpatentable
35 U.S.C. § 318

IPR2022-00042
IPR2022-00045
Patent 10,875,007 B2

I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review of claims 1–14, 16–32, and 34–37 (“the challenged claims”) of U.S. Patent No. 10,875,007 B2 (Ex. 1001, “the ’007 patent”). We have jurisdiction under 35 U.S.C. § 6, and enter this Final Written Decision pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons set forth below, we determine that JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) has shown, by a preponderance of the evidence, that some of the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e).

A. *Consolidated Proceedings*

The two captioned proceedings (IPR2022-00042 and IPR2022-00045 (or “the ’045 IPR”)) involve the ’007 patent and challenge the same set of claims. The asserted grounds and prior art contentions are different in each proceeding. Consolidation is appropriate where, as here, the Board can more efficiently handle the common issues and evidence, and also remain consistent across proceedings. Under 35 U.S.C. § 315(d), the Director may determine the manner in which these pending proceedings may proceed, including “providing for stay, transfer, consolidation, or termination of any such matter or proceeding.” *See also* 37 C.F.R. § 42.4(a) (“The Board institutes the trial on behalf of the Director.”). There is no specific Board rule that governs consolidation of cases. But 37 C.F.R. § 42.5(a) allows the Board to determine a proper course of conduct in a proceeding for any situation not specifically covered by the rules and to enter non-final orders to administer the proceeding. Therefore, on behalf of the Director under § 315(d), and for a more efficient administration of these proceedings, we

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consolidate IPR2022-00042 and IPR2022-00045 for purposes of rendering this Final Written Decision.

B. *Evidence*

Petitioner relies upon information that includes the following:

Ex. 1004, M. Linhult, *et al.*, *Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach*, 55 PROTEINS: STRUCTURE, FUNCTION, AND BIOINF., 407–16 (2004) (“Linhult”).

Ex. 1005, L. Abrahmsén, *et al.*, U.S. Patent No. 5,143,844 (issued Sept. 1, 1992) (“Abrahmsén”).

Ex. 1006, S. Hober, PCT Publication No. WO 03/080655 A1 (published Oct. 2, 2003) (“Hober”).

Ex. 1018, H. Berg., U.S. Patent Application Publication No. 2006/0134805 (published June 22, 2006) (“Berg”).

C. *Procedural History*

Petitioner filed a Petition for an *inter partes* review of the challenged claims under 35 U.S.C. § 311. Paper 1¹ (“Pet.”). Petitioner supported the Petition with the Declaration of Dr. Steven M. Cramer. Ex. 1002. Cytiva Bioprocess R&D AB (“Patent Owner”) filed a Patent Owner Preliminary Response to the Petition. Paper 8.

On May 19, 2022, pursuant to 35 U.S.C. § 314(a), we instituted trial (“Decision” or “Dec.” (Paper 10)) to determine whether any challenged claim of the ’007 patent is unpatentable.

¹ We note that the evidence filed in both proceedings is generally consistent in having the same exhibit number. Therefore, we reference exhibits and paper numbers as they appear in the record of IPR2022-00042, unless otherwise noted.

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In IPR2022-00042, Petitioner asserts the following grounds of unpatentability (Pet. 4):

Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–11, 20–29	103(a)	Linhult, Abrahmsén
1–14, 16–32, 34–37	103(a)	Linhult, Hober
1–14, 16–32, 34–37	103(a)	Linhult, Abrahmsén, Hober
1–14, 16–32, 34–37	103(a)	Abrahmsén, Hober

In IPR2022-00045, Petitioner asserts the following grounds of unpatentability ('045 IPR Pet. 4):

Claim(s) Challenged	35 U.S.C. §	Reference(s)/Basis
1–14, 16–18, 20–32, 34–36	103(a)	Berg, Linhult
4–8, 10, 19, 22–26, 28, 37	103(a)	Berg, Linhult, Hober
1–3, 9, 10, 12–14, 16–18, 20, 21, 27, 28, 30–32, 34–36	103(a)	Berg, Abrahmsén
4–8, 10, 11, 19, 22, 26, 28, 29, 37	103(a)	Berg, Abrahmsén, Hober

Patent Owner filed a Patent Owner Response to the Petition. Paper 15 (“PO Resp.”). Patent Owner supported the Response with the Declaration of

² The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the '007 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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Dr. Daniel Bracewell (Ex. 2025). *See* PO Resp., iv (Exhibit List). Petitioner filed a Reply to the Patent Owner Response. Paper 28 (“Reply”). Petitioner supported the Reply with a Reply Declaration from Dr. Steven M. Cramer. Ex. 1061. Patent Owner filed a Sur-reply to Petitioner’s Reply. Paper 34 (“Sur-reply”).

On February 16, 2023, the parties presented arguments at an oral hearing. Paper 35. The hearing transcript has been entered in the record. Paper 39 (“Tr.”).

For the reasons set forth below, we determine that Petitioner has shown by a preponderance of the evidence that claims 1–10, 12–14, 16–28, 30–32, and 34–37 of the ’007 patent are unpatentable, but find that Petitioner has not shown by a preponderance of the evidence that claims 11 and 29 are unpatentable.

D. *Related Matters*

The ’007 patent is at issue in *Cytiva Bioprocess R&D et al. v. JSR Corp. et al.*, Case No. 21-310-RGA (D. Del.). Pet. 2; Paper 5, 1.

In addition to the ’007 patent, Petitioner filed Petitions for *inter partes* review of related U.S. patents as follows: U.S. Patent No. 10,343,142 B2 (“the ’142 patent”) in IPR2022-00041 and IPR2022-00044; and U.S. Patent No. 10,213,765 B2 (“the ’765 patent”) in IPR2022-00036 and IPR2022-00043. Pet. 2–3; Paper 5, 1–2. The ’007 patent is a continuation of the ’142 patent which is a continuation of the ’765 patent. Ex. 1001, code (60).

E. *Subject matter background*

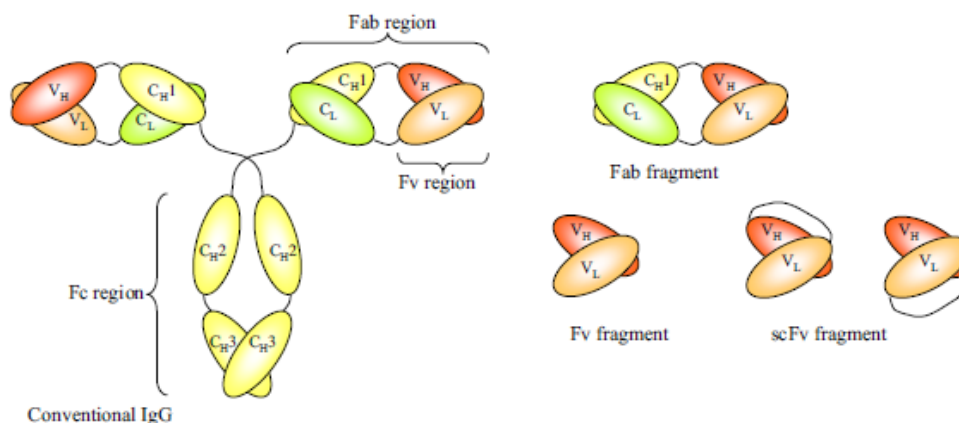
Antibodies (also called immunoglobulins) are glycoproteins, which specifically recognize foreign molecules. These recognized foreign

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molecules are called antigens. Ex. 2001, 1. A schematic representation of the structure of a conventional IgG and fragments is shown below:



The figure (Ex. 2001, 2 (Fig. 1)), reproduced above, shows

the structure of a conventional IgG and fragments that can be generated thereof. The constant heavy-chain domains CH1, CH2 and CH3 are shown in yellow, the constant light-chain domain (CL) in green and the variable heavy-chain (VH) or light-chain (VL) domains in red and orange, respectively. The antigen binding domains of a conventional antibody are Fabs and Fv fragments. Fab fragments can be generated by papain digestion. Fvs are the smallest fragments with an intact antigen-binding domain. They can be generated by enzymatic approaches or expression of the relevant gene fragments (the recombinant version). In the recombinant single-chain Fv fragment, the variable domains are joined by a peptide linker. Both possible configurations of the variable domains are shown, i.e. the carboxyl terminus of VH fused to the N-terminus of VL and vice versa.

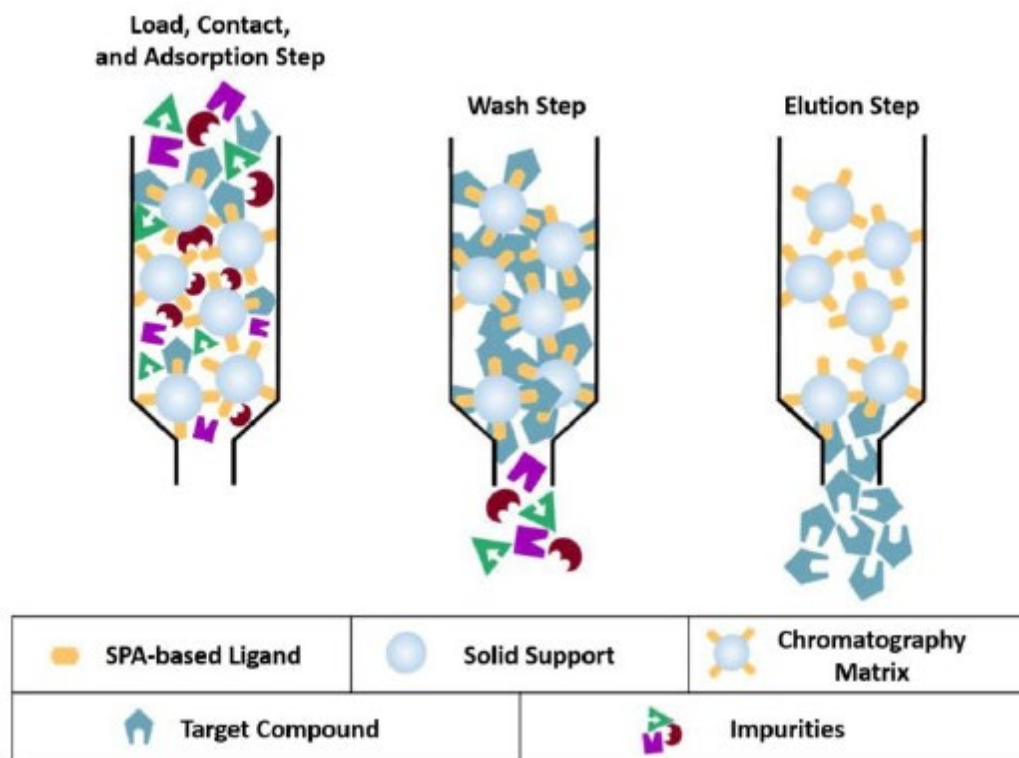
Ex. 2001, 2; *see also* PO Resp. 5.

Below is a generic, exemplary schematic that shows how affinity purification typically works:

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The figure shows the schematic of the loading, contact, and adsorbing step onto a column, followed by the wash step, and finally the elution and collection of the target compound. Ex. 1002 ¶ 24 (citing Ex. 1014 §§ 1.1, 4.2.); *see also* PO Resp. 7 (“In a typical process, the composition containing the desired antibody then is loaded onto (i.e., pumped or injected into) the column.”); Pet. 6; *see generally* Ex. 1014.

F. *The '007 patent (Ex. 1001)*

The '007 patent discloses an affinity ligand useful for isolating antibodies or antibody fragments. *See* Ex. 1001, Abstr., 1:43–49. Affinity ligands were previously used to capture antibodies (immunoglobulin proteins) in chromatography matrices. *See id.* at 2:1–19. After each use, chromatography matrices are cleaned using an alkaline protocol “known as Cleaning In Place (CIP).” *See id.* at 2:20–54. CIP damages protein-based

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affinity ligands through deamidation and cleavage of peptides. *Id.* at 2:35–54.

The '007 discloses prior efforts to genetically engineer protein-based affinity ligands to improve stability to alkaline cleaning, e.g., CIP. *See id.* at 2:53–54; 3:3–55. Specifically, the '007 patent describes a modified affinity ligand based on *Staphylococcus* protein A (“SpA”). SpA was “widely used” as an affinity chromatography ligand due to its ability to bind to antibodies without affecting the antibodies’ ability to bind to antigens. *Id.* at 2:55–68. However, the '007 patent discloses that unmodified SpA required milder cleaning conditions than conventional CIP to prevent damaging the SpA ligand. *Id.* at 3:18–25. Accordingly, the '007 patent describes a need for modifying SpA to improve alkaline resistance while maintaining binding selectivity. *Id.* at 3:25–28.

The '007 patent describes prior efforts to modify SpA through its constituent domains. *See id.* at 3:42–55. SpA is composed of five domains, designated as domains E, D, A, B, and C, which are able to bind to antibodies at the antibodies’ fragment crystallizable (“Fc”) region. *Id.* at 2:60–65. The '007 patent describes a known modified SpA B-domain with “increased chemical stability at pH-values of up to about 13–14.” *Id.* at 3:42–55 (citing WO 03/080655). The increased stability results from mutating “at least one asparagine residue . . . to an amino acid other than glutamine or aspartic acid,” as it was known that asparagine and glutamine residues were sensitive to deamidation and cleavage in alkaline conditions. *See id.* at 2:42–47; 3:42–55.

Against this background, the '007 patent discloses an affinity ligand based on SpA domain C that “is capable of withstanding repeated cleaning-

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in-place cycles.” *Id.* at 4:9–13. The Specification discloses that “Domain C ligand, which contains as many as six asparagine residues, was not . . . expected to present any substantial alkaline-stability as compared to protein A.” *Id.* at 5:54–56. The asparagine residues are shown in the amino acid sequence of wild-type SpA Domain C SEQ ID NO 1. *Id.* at 6:40–43; 15:1–25. The Specification describes “a specific embodiment [of] the chromatography ligand according to the invention [that] comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A.” *Id.* at 6:56–61. In other words, the modified SpA Domain C includes alanine (A) instead of glycine (G) at position 29 of the amino acid sequence. *See id.* at 15:52–54. The ’007 patent further describes a multimeric chromatography ligand including at least two Domain C units, or functional variants thereof. *Id.* at 7:35–38.

1. Illustrative Claims

Claims 1 and 20 are the independent claims, reproduced below, and are illustrative of the claimed subject matter of the ’007 patent.

1. A process for isolating one or more target compound(s), the process comprising:

- (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 52 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G)

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at a position corresponding to position 29 of SEQ ID NO. 1; and

- (b) adsorbing the target compound(s) to the ligand;
- (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand; and,
- (d) performing a cleaning in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.

20. A process for isolating one or more target compound(s), the process comprising:

- (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids in alignment with SEQ ID NO. 1, and wherein each polypeptide has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1;
- (b) adsorbing the target compound(s) to the ligand; and,
- (d) performing a clean in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.

Ex. 1001, 15:40–61, 17:22–40

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II. ANALYSIS

A. *Principles of Law*

“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify “with particularity . . . the evidence that supports the grounds for the challenge to each claim”)). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner must demonstrate by a preponderance of the evidence³ that the claims are unpatentable. 35 U.S.C. § 316(e); 37 C.F.R. § 42.1(d). A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time of the invention to a person having ordinary skill in the art. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations, including “the scope and content of the prior art”; “differences between the prior art and the claims at issue”; “the level of ordinary skill in the art;” and “objective evidence of non-obviousness.” *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

³ The burden of showing something by a preponderance of the evidence requires the trier of fact to believe that the existence of a fact is more probable than its nonexistence before the trier of fact may find in favor of the party who carries the burden. *Concrete Pipe & Prods. of Cal., Inc. v. Constr. Laborers Pension Tr. for S. Cal.*, 508 U.S. 602, 622 (1993).

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In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. *Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner asserts that a person of ordinary skill in the art would have had

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(1) at least an advanced degree (*e.g.*, a Master's or Ph.D.) in biochemistry, process chemistry, protein chemistry, chemical engineering, molecular and structural biology, biochemical engineering, or similar disciplines; (2) several years of post-graduate training or related experience (including industry experience) in one or more of these areas; and (3) an understanding of the various factors involved in purifying proteins using chromatography.[] Such a person would have had multiple years of experience with affinity ligand design and protein purification.

Pet. 9–10 (citing Ex. 1002 ¶¶ 13–14). Patent Owner does not dispute Petitioner's definition of the person of ordinary skill. *See generally* PO Resp. Because Petitioner's proposed definition is unopposed and appears consistent with the Specification and art of record, we apply it here.

C. *Claim Construction*

The Board applies the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.200(b) (2021). Under that standard, claim terms “are generally given their ordinary and customary meaning” as understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc).

Claims 1 and 20 recite “at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides.” Ex. 1001, 15:46–49; 17:28–31. Petitioner argues that “‘the ligand comprising at least two polypeptides’ refers to a multimeric ligand (such as a tetramer) comprised of multiple polypeptides, each of which is a monomer.” Pet. 19–20 (citing Ex. 1002 ¶¶ 43–45). Petitioner argues that this construction is consistent with Patent

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Owner's implicit construction in the Delaware litigation between the parties.

Id.

Patent Owner does not challenge Petitioner's claim construction. *See generally* PO Resp.

According to the Specification, "the present invention . . . relates to a multimeric chromatography ligand (also denoted a 'multimer') comprised of at least two domain C units, or a functional fragments [sic] or variants thereof." Ex. 1001, 7:35–38. The Specification additionally recites that a multimer containing only domain C units can, however, include linkers. *Id.* at 7:39–41. In addition, the Specification describes that "the multimer comprises one or more additional units, which are different from Domain C." *Id.* at 7:52–53. Based on these disclosures in the Specification, a multimer is composed of at least two or more monomers. Because Petitioner's construction is consistent with the '007 patent's express construction of the term, we apply that construction for the purposes of this decision.

Having considered the parties' positions and evidence of record, we determine that the express construction of any other claim term is unnecessary to resolve the disputed issues in this matter. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))). To the extent further discussion of the meaning of any claim term is necessary to our decision, we provide that discussion below in our analysis of the asserted grounds of unpatentability.

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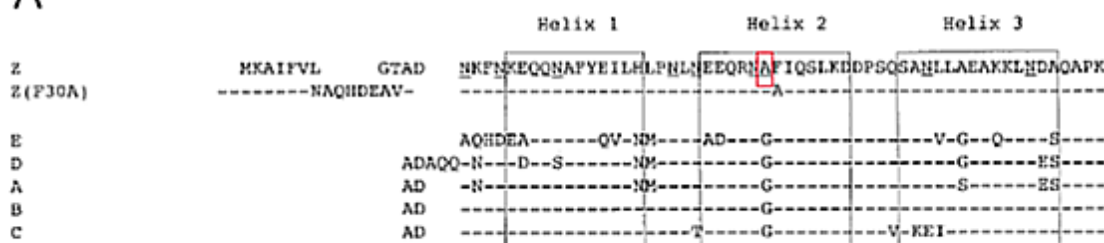
D. *Overview of Asserted References*1. *Linhult (Ex. 1004)*

Linhult is titled “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach.”

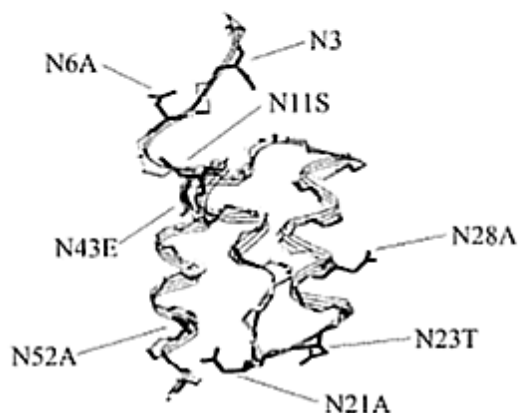
Ex. 1004, 1. Linhult discloses that due to the high affinity and selectivity of Staphylococcal protein A (SPA), “it has a widespread use as an affinity ligand for capture and purification of antibodies” but that “it is desirable to further improve the stability in order to enable an SPA-based affinity medium to withstand even longer exposure to the harsh conditions associated with cleaning-in-place (CIP) procedures.” *Id.*, Abstr. Linhult discloses, “[t]o further increase the alkaline tolerance of SPA, we chose to work with Z, which is a small protein derived from the B domain of SPA.” *Id.* at 2.

Figures 1A and 1B of Linhult are reproduced below.

A



B



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Figure 1A shows “[a]mino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C)” in which the horizontal lines indicate amino acid identity and “one glycine in the B domain [is] replaced [and] underlined” as annotated by the Board with a red box. *Id.* “Z(F30A), and all mutants thereof includes the same N-terminal as Z(F30A)” and “Z(N23T) was constructed with the same N-terminal as Z.” *Id.*⁴ Figure 1B shows “[t]he three-dimensional structure of the Z domain” and “the different substitutions are indicated.” *Id.* Specifically, Linhult discloses,

[t]he B domain has been mutated in order to achieve a purification domain resistant to cleavage by hydroxylamine. An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine.[] Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.[] Protein Z is well characterized and extensively used as both ligand and fusion partner in a variety of affinity chromatography systems.

Id. Using a 0.5 M NaOH cleaning agent and “a total exposure time of 7.5 h for Z(F30A) and mutants thereof,” Linhult determines that “N23 seems to be very important for the functional stability after alkaline treatment of Z(F30A)” and “Z(F30A, N23T) shows only a 28% decrease in capacity despite the destabilizing F30A-mutation.” *Id.* at 410–11; Figs. 2, 3. Linhult reports that “[h]ence, the Z(F30A, N23T) is almost as tolerant as Z and is thereby the most improved variant with Z(F30A) as scaffold.” *Id.* at 411; Figs. 2, 3.

⁴ The mutation N23T having a change in amino acid correlates with the amino acid N next to the “Helix 2” box of Figure 1A as annotated by Petitioner. *See* Pet. 12.

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Linhult further discloses that “Z, Z(F30A), and mutated variants were covalently coupled to HiTrap™ affinity columns,” that “[t]he Z domain includes 8 asparagines (N3, N6, N11, N21, N23, N28, N43, and N52; Fig. 1),” and that “since the amino acid is located outside the structured part of the domain, it will most likely be easily replaceable during a multimerization of the domain to achieve a protein A-like molecule.” *Id.* at 410. Linhult confirms that “the affinity between Z(F30A) and IgG was retained despite the mutation.” *Id.* In Linhult’s studies, “[h]uman polyclonal IgG in TST was prepared and injected onto the columns in excess” and “[a] standard affinity chromatography protocol was followed.” *Id.*

2. *Abrahmsén (Ex. 1005)*

Abrahmsén “relates to a recombinant DNA fragment coding for an immunoglobulin G ([I]gG) binding domain related to staphylococcal protein A . . . and to a process for cleavage of a fused protein expressed by using such fragment or sequence.” Ex. 1005, 1:8–13. Abrahmsén discloses that “[b]y making a gene fusion to staphylococcal protein A any gene product can be purified as a fusion protein to protein A and can thus be purified in a single step using IgG affinity chromatography.” *Id.* at 1:22–26. Abrahmsén explains that Protein A has “5 Asn-Gly in the IgG binding region of protein A” which “makes the second passage through the column irrelevant as the protein A pieces released from the cleavage will not bind to the IgG.” *Id.* at 1:58–63. Abrahmsén provides a solution to this problem “by adapting an IgG binding domain so that no Met and optionally no Asn-Gly is present in the sequence.” *Id.* at 1:64–67.

Abrahmsén discloses that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.”

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Id. at 2:21–23. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén discloses that the recombinant DNA fragment can “cod[e] for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.” *Id.* at 2:32–37. According to Abrahmsén, from a simulation of the Gly to Ala amino acid change in the computer, it was “concluded that this change would not interfere with folding to protein A or binding to IgG.” *Id.* at 5:13–16.

3. *Hober (Ex. 1006)*

Hober “relates to . . . a mutant protein that exhibits improved stability compared to the parental molecule” and “also relates to an affinity separation matrix, wherein a mutant protein according to the invention is used as an affinity ligand.” Ex. 1006, 1. Hober discloses that removal of contaminants from the separation matrix involves “a procedure known as cleaning-in-place (CIP)” but “[f]or many affinity chromatography matrices containing proteinaceous affinity ligands,” the alkaline environment “is a very harsh condition and consequently results in decreased capacities owing to instability of the ligand.” *Id.* at 1–2. According to Hober, stability to alkaline conditions can be engineered into a protein. *Id.* at 2. To improve the stability of a Streptococcal albumin-binding domain (ABD) in alkaline environments, it has been reported to involve the role of peptide conformation in the rate and mechanism of deamidation of asparaginyl residues and that “the shortest deamidation half time have been associated

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with the sequences -asparagine-glycine and -asparagine-serine.” *Id.* at 2.

Further, from a study of a mutant of ABD that was created, it was concluded that “all four asparagine residues can be replaced without any significant effect on structure and function.” *Id.* at 2–3. Hober points out that the staphylococcal protein A (SPA) contains domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species and reagents of this protein with their high affinity and selectivity have found a widespread use in the field of biotechnology. *Id.* at 3. Accordingly, “there is a need in this field to obtain protein ligands capable of binding immunoglobulins, especially via the Fc-fragments thereof, which are also tolerant to one or more cleaning procedures using alkaline agents.” *Id.* at 4.

In one embodiment of Hober, a multimer “comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A” in which “asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids” for advantageous structural stability reasons wherein “the glycine residue in position 29 of SEQ ID NO: 1 has also been mutated, preferably to, an alanine residue.” *Id.* at 12. Hober’s SEQ ID NO: 1 and is reproduced below.

```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1          5          10          15

Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln
          20          25          30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
          35          40          45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50          55

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Id. at SEQUENCE LISTING 1. SEQ ID NO: 1 shows a domain of *Staphylococcus aureus* having Glycine (Gly) as an amino acid at the position 29, as annotated by the Board via red highlighting.

Hober further discloses that its matrix for affinity separation “comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which [in the] protein at least one asparagine residue has been mutated to an amino acid other than glutamine.” *Id.* at 13. For its method of isolating an immunoglobulin, Hober discloses “in a first step, a solution comprising the target compounds, . . . is passed over a separation matrix under conditions allowing adsorption of the target compound to ligands present on said matrix” and “[i]n a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption, i.e. release of the target compound.” *Id.* at 13.

E. *Obviousness in view of Linhult, Abrahamssén, and Hober*

1. *Petitioner’s Contentions*

a) *Claims 1, 2, and 20*

Petitioner contends that “*Linhult* describes the common use of chromatography matrices in the biotechnology field, and, more specifically, SPA-based chromatography matrices to isolate target compounds.” Pet. 16 (citing Ex. 1002 ¶ 83). Petitioner contends that “*Linhult* describes a process whereby a ‘[h]uman polyclonal IgG in TST^[5]’ was prepared and injected onto the columns in excess,’ ‘[a] standard affinity chromatography protocol was followed,’ and ‘eluted material was detected.’” Pet. 17 (citing Ex. 1004,

⁵ TST is a solution containing 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20. Ex. 1004, 4.

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4). Petitioner contends that a person of ordinary skill in the art “would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of loading a liquid comprising the target compound onto the column, thereby allowing the liquid to contact the recited SPA-based chromatography matrix.” *Id.* (citing Ex. 1002 ¶¶ 88–89). In other words, Petitioner contends that the contacting step is a well-known step in the field of affinity chromatography.

Petitioner contends that “[a]dsorbing target compounds to SPA-based ligands coupled to the solid supports was a well-known and conventional feature of SPA-based affinity chromatography.” Pet. 23–24 (citing Ex. 1002 ¶ 122, *see also id.* ¶¶ 120–121; Ex. 1004, 4).

Petitioner contends that “a [person of ordinary skill in the art] would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of eluting target compounds from SPA-based ligands coupled to the solid supports in a chromatography matrix.” Pet. 24 (citing Ex. 1002 ¶ 124, *see also id.* ¶¶ 121–125; Ex. 1004 ¶ 4). Petitioner, therefore contends that it is well-known that a standard affinity chromatography protocol contains three active steps: (1) contacting, (2) adsorbing, and (3) eluting.

Petitioner contends that Linhult teaches a chromatography matrix. Pet. 18. Specifically, Linhult teaches using a HiTrap chromatography affinity column made up of agarose beads that serve as a solid support for “coupling SPA-based ligands.” Pet. 18 (citing Ex. 1004, 4; Ex. 1002 ¶ 98). “*Linhult* discloses that its SPA-based ligands were ‘coupled to’ the solid support agarose beads [] contained in HiTrap™ affinity columns.” *Id.* at 18 (citing Ex. 1004, 4). Petitioner contends that “Linhult discloses that

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‘multimerization’ of SPA monomers is performed to ‘achieve’ an ‘[SPA-]like’ affinity ligand.” *Id.* at 19 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 103–106). Petitioner contends that “Figure 1(a), *Linhult* describes at least 55 amino acids of SPA’s naturally-occurring C domain (i.e., SEQ ID NO. 1).” *Id.* (citing Ex. 1004, 1, Fig. 1(a); *see* Ex. 1005, Fig. 2; Ex. 1006, Fig. 1; Ex. 1008, 639, Fig. 1). Petitioner contends “that all ‘five SPA domains show individual affinity for the Fc-fragment . . . as well as certain Fab-fragments of [antibodies] from most mammalian species.” *Id.* at 20 (citing Ex. 1004, 1).

Petitioner contends that it was “known that individual SPA domains, including the C domain, could be used to construct SPA-based affinity ligands for purifying proteins.” *Id.* at 20 (citing Ex. 1002. ¶¶ 29, 113; Ex. 1004, 1; Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). Petitioner contends that *Linhult* teaches a person of ordinary skill in the art “that avoiding the Asn₂₈-Gly₂₉ dipeptide sequence through a G29A mutation, including on the C domain, would yield an SPA-based ligand having increased alkali-stability.” *Id.* at 21 (citing 1002 ¶¶ 112–116; Ex. 1011; Ex. 1012; Ex. 1013). Petitioner acknowledges that “*Linhult* does not expressly disclose a C(G29A)-based SPA ligand,” but asserts that “[r]egardless, it would have been obvious to a [person of ordinary skill in the art] to modify *Linhult* based on the teachings of *Abrahmsén* to incorporate a C(G29A)-based SPA ligand in a chromatography matrix.” *Id.* at 22 (Ex. 1002 ¶¶ 111–120). Petitioner contends “*Abrahmsén* expressly discloses ‘a recombinant DNA coding for **any of** the E D A B C domains of [SPA], wherein the glycine codon(s) in the Asn_[28]-Gly_[29] coding constellation has

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been replaced by an alanine codon.”’ *Id.* at 22 (bracketing in and emphasis in original) (citing Ex. 1005, 2:32–37).

A [person of ordinary skill in the art] would have had good reason to combine the teachings from *Abrahmsén* with *Linhult* because a G29A mutation was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence, i.e., the “most sensitive amino acid sequence to alkaline conditions,” such as those used in CIP. (Ex. 1002 ¶¶ 119, 135; Ex. 1004, [2].) Moreover, a [person of ordinary skill in the art] would have been drawn to a C-domain-based ligand, which, as *Linhult* describes, shows individual affinity for antibodies and already includes 23T (as well as 43E), which it disclosed as providing “remarkably increased” stability. (Ex. 1002 ¶¶ 115-16; Ex. 1004, [1], [8–9].)

Pet. 23.

Applying the teachings of *Abrahmsén* with *Linhult* would have involved merely combining known elements in the field (e.g., a process for isolating one or more target compounds using an affinity chromatography matrix comprising a G29A-containing ligand coupled to a solid support, as in *Linhult*, and a C(G29A)-based amino acid sequence, as in *Abrahmsén*) according to known ligand-construction methods to yield a predictable result[] (e.g., a process for isolating one or more target compounds using the recited affinity chromatography matrix). (Ex. 1002 ¶136.) *See, e.g., KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 415-21 (2007); *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1239-40 (Fed. Cir. 2010).

Pet. 25–26. Petitioner further contends “*Hober*’s disclosure is in the context of SPA-based affinity chromatography utilizing G29A-containing ligands, and, in fact, further confirms that the teachings of *Abrahmsén* are applicable in this context.” Pet. 52 (citing Ex. 1002 ¶¶ 260–275; Ex. 1006, 10–12); Ex. 1006, 12 (“the present multimer also comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A. . . . for structural stability

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reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to an alanine residue”).

b) Claims 11 and 29

Petitioner contends that “[t]he capability of ‘bind[ing] to the Fab part of an antibody,’ as recited in claims 11 and 29, is an inherent property of the recited C(G29A)-based SPA ligand.” Pet. 30 (citing Ex. 1002 ¶¶ 155–162).

c) Claims 3–10, 12–14, 16–19, 21–28, 30–32, and 34–37

With respect to claims 3–10, 12–14, 16–19, 21–28, 30–32, and 34–37, Petitioner directs our attention to where in the asserted art of record the various limitations of the dependent claims may be found. *See* Pet. 20 n.13, 26–30, 37–41.

2. Patent Owner’s Contentions

Patent Owner argues that the Petition fails to demonstrate that there would have been motivation and a reason to make and use the chromatography matrix as claimed (PO Resp. 17–48); that the Petition has not established that there is a reasonable expectation of success in arriving at the claimed matrix (*id.* at 49–54); that the art teaches away from making the G29A modification (*id.* at 38–44); that the artisan would have been motivated make additional mutations (*id.* at 44–48).

a) Matrix

According to Patent Owner, “Petitioners fail to explain *why* the POSA would have been motivated to select Domain C’s amino acid sequence as the foundation for an engineered SPA ligand with favorable properties.”

PO Resp. 19. Specifically arguing that the obviousness analysis requires the

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prior art be viewed as a whole. PO Resp. 21 (citing *In re Wesslau*, 353 F.2d 238 (CCPA 1965); *In re Enhanced Sec. Rsch., LLC*, 739 F.3d 1347, 1355 (Fed. Cir. 2014); *Impax Lab 'ys Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018)). In other words, because obviousness requires considering the prior art as a whole and no one was working on domain C at the time of the invention, Patent Owner asserts, it would not have been obvious to select domain C for further development or genetic modification.

Patent Owner argues that because nobody was working on domain C at the time the invention was filed, therefore, the selection of domain C for further development could not possibly be obvious. *See* PO Resp. 24 (“Reliance on *KSR* also is foreclosed by the evidence that no one in the art was seeking to modify Domain C.”), *see also id.* at 25 (“But no prior art cited by Petitioners singles Domain C out for further development. Ex. 2025 ¶¶ 89–96”), *id.* at 27 (“Dr. Cramer [Petitioner’s expert] himself highlights, it would have been natural for the POSA to further develop the domain—Domain B—that was best understood and for which there was a crystal structure available. Ex. 1002 ¶ 33; Ex. 2015 at 137:20–138:19; Ex. 2017”), *id.* at 28–29 (“The notion that this body of work would lead the POSA to discard the improved ligands the references themselves focus on, and instead start experimenting with mutations to Domain C—strains credulity. Ex. 2025 ¶¶ 92–95”).

According to Patent Owner, neither Linhult nor Abrahmsén supply the motivation to start with domain C. “Linhult focuses exclusively on, and concerns improvements to, the alkaline stability of Domain Z by mutating asparagine residues. *See* Ex. 2025 ¶¶ 64–67, 92.” PO Resp. 30. “Rather than use Domain C, the POSA reviewing Linhult would be motivated to keep

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working with Domain Z, adopting the N23T mutation. Ex. 2025 ¶ 67 & n.3.”

PO Resp. 31. “Neither Abrahmsén itself nor the Petition provide any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domains. *WBIP[LLC v. Kohler Co.]*, 829 F.3d 1317, 1337 (Fed. Cir. 2016).” PO Resp. 31–32.

b) Reasonable Expectation of Success

Patent Owner argues that “the field of protein engineering is notoriously unpredictable.” PO Resp. 22 (citing Ex. 2025 ¶¶ 50–52). Arguing that “despite their supposed structural similarity, there are a number of differences between the naturally-occurring domains of protein A, including five different amino acids in the sequences of Domain B (with which the industry was quite familiar) and Domain C (which remained virtually ignored as of the priority date).” *Id.* at 22–23 (citing Ex. 2025 ¶ 48).

Protein engineering is a highly complex and unpredictable field and was all the more so as of the priority date more than fifteen years ago. *See, e.g.,* Ex. 2025 ¶¶ 50-52. . . . As amply demonstrated by the effect of the G29A mutation on Domain Z’s Fab-binding ability, even a single amino acid substitution can drastically alter the properties of a protein. Ex. 2025 ¶ 52; Ex. 2015 at 51:15-52:1 ([Dr. Cramer, Petitioner’s expert] agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability), 18:10-12, 73:16-20.

PO Resp. 35–36.

Patent Owner argues that

The Federal Circuit has rejected arguments premised on the notion that a homologous structure renders an invention obvious, particularly given the difficulty and uncertainty in the art as of the priority date. *See, e.g., Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208 (Fed. Cir. 1991) (holding the use of a monkey gene to probe for a roughly 90 percent “homologous”

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human gene would not have been obvious, particularly given expert testimony that isolating a particular gene would have been “difficult” and the lack of certainty in the endeavor).

PO Resp. 37–38. Specifically, Patent Owner argues that the Fab-binding capability of a ligand could not have been predicted and therefore there is no reasonable expectation of success in using the ligand in a process of purifying a target compound. *See* PO Resp. 49–55.

c) Teaching Away

Patent Owner argues that the prior art would have told the person of ordinary skill in the art to avoid a G29A a modification to domain C.

PO Resp. 39–44. In other words, it’s Patent Owner’s contention that the prior art teaches away from making this modification. “The very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding ‘negligible’ when implemented in Domain B.” PO Resp. 41. Patent Owner argues that a person seeking to improve Fab binding would have avoided a G29A substitution of domain C. PO Resp. 41–44.

d) Additional Modifications

Patent Owner argues that “the prior art would have taught the POSA to make asparagine substitutions, not glycine substitutions, to address alkaline stability concerns.” PO Resp. 44–45. In other words, Patent Owner argues the prior art would have suggested making additional substitutions most notably in the asparagine residues of domain C. *Id.* at 46.

3. Petitioner’s Reply

In response, Petitioner argues that

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Abrahmsén and *Hober* each expressly pointed to a C(G29A) mutation (Ex. 1005, 2:32-37; Ex. 1006, 12), which was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence (*see, e.g.*, Ex. 1004, [2]). As the Board recognized, “*Abrahmsén* provides motivation for making [the G29A] mutation in **any of the IgG binding domains.**” (Decision, 26; *see also* Ex. 1057, 97:3-16 (Dr. Bracewell admitting that *Abrahmsén* discloses a G29A mutation to any of the five domains, including Domain C).)

Reply 2.

A POSA would have reasonably expected success in combining these teachings to achieve the claimed affinity chromatography matrix given the well-known fact that each individual domain, including Domain C, has affinity for antibodies (Ex. 1004, [1]), as well as *Abrahmsén*’s confirmation that G29A “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1057, 99:13-101:21 Ex. 1005, 5:13-16; Ex. 1002 ¶131).

Id. at 3.

Petitioner argues that Patent Owner “has not disputed that *Abrahmsén* disclosed that G29A ‘would not interfere with folding to protein A or binding to IgG.’ (Ex. 1005, 2:32–37, 5:4–16; Ex. 1057, 109:20–110:17.) Nor does it take issue with its own statements in *Hober* that G29A is advantageous for ‘structural stability reasons.’ (Ex. 1006, 12.)” *Id.* at 9. Petitioner contends that Patent Owner’s lack of binding argument is contradicted by “*Abrahmsén* and *Hober*, which make clear that G29A does not affect the ability of an SPA ligand to bind to an antibody. (Ex. 1005, 2:32–37, 5:4–16; Ex. 1006, 12.)” *Id.* at 10.

Petitioner argues that “a POSA would have started with any one of the naturally occurring domains. (Decision, 26-28.) To then increase alkali stability, a POSA would have made the simplest, well-known substitution: G29A. (Section II.A.1–2; Ex. 1061 ¶¶8–15.)” Reply 11.

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Petitioner argues “that Fab-binding was an inherent feature of a C(G29A)-based ligand—which [Patent Owner] does not appear to dispute. (Decision, 34; [Prelim.] Resp., 53–54.) In fact, [Patent Owner] acknowledges that ‘C(G29A)-based SPA ligands retained substantial Fab-binding ability.’ (Resp., 56–57.)” *Id.* at 14.

Petitioner argues that “Fab-binding is not being used [in the Petition] as part of a finding of a motivation to combine; rather, it is an inherent property [of the composition] being claimed. And necessarily present properties do not add patentable weight when they are claimed as limitations. *In re Kubin*, 561 F.3d 1351, 1357 (Fed. Cir. 2009).” Reply 15–16. Petitioner further argues that Patent Owner’s reliance on *Honeywell* is misplaced because “*Honeywell* had to do with an inherent property being used as a teaching in an obviousness analysis; it did not involve a limitation in the challenged claim reciting an inherent property.” Reply 15 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. De C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017); *see also Pernix Ireland Pain v. Alvogen Malta Operations*, 323 F. Supp. 3d 566, 607(D. Del. 2018)).

4. Patent Owner’s Sur-reply

Patent Owner argues that “Petitioners, and the Institution Decision, overlook an important point of consensus between the parties’ experts: the field of protein engineering is notoriously *unpredictable*.” Sur-reply 2. Patent Owner maintains that Petitioner has not identified a motivation to start from Domain C. *Id.* at 3. Patent Owner argues that “Petitioners would have the Board look past the multitude of references teaching a preference for Domains B and Z—including Petitioners’ foundational references—and seize upon fleeting mentions of Domain C.” *Id.* at 7 (citing *In re Wesslau*,

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353 F.2d 238, 241 (C.C.P.A. 1965)”). Patent Owner argues that “[m]ere sequence homology does not make the field predictable, as both experts observe, Ex. 2015 at 51:15-52:1, 56:4-12, 75:12-22, 73:16-20; Ex. 2025 ¶¶ 50-52; Ex. 2049 at 72:1-73:12, and as the vastly different Fab-binding properties of the near-identical Domains B and Z well illustrate, Ex. 2029 at 8.” *Id.* at 8–9.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7–10.” Sur-reply 11.

5. Analysis

a) Claims 1, 2, and 20

Independent claims 1, 2, and 20 of the ’007 patent are directed to a method of isolating a target compound using a chromatography matrix composition. Ex. 1001, 15:40–64, 17:22–40. The claims recite three active steps: (1) contacting, (2) adsorbing, (3) eluting the target compound from the chromatography matrix, and (4) cleaning the chromatography matrix in place using a NaOH solution. *Id.* Claim 1 further stipulates that the chromatography matrix composition (a solid support) has the following features: a ligand is attached to the matrix and the ligand is made up of at least two polypeptides comprising 52 contiguous amino acids of SEQ ID NO: 1⁶ each having a G29A mutation. Claim 2 depends from claim 1 and

⁶ Wild type amino acid sequence of domain C from *Staphylococcus* protein A (SPA). *See* Ex. 1001, 4:27, 6:35–36, 6:51–52.

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recites “wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1.” *Id.* at 15:62–64; Pet. 20 n.13. Claim 20 is similar to claim 1, except that claim 20 recites “at least 55 amino acids in alignment with SEQ ID NO. 1” instead of “at least 52 contiguous amino acids of modified SEQ ID NO. 1” as recited in claim 1, and does not recite the eluting step. *Id.* at 17:22–40.

(1) *Method*

Claims 1, 2, and 20 are directed to a method of using a particular matrix. The method comprises two parts: (a) the process steps and (b) the structure of the matrix.

(a) *Process Steps*

Petitioner asserts that the combination of Linhult, Abrahmsén, and Hober teaches or suggests the standard affinity chromatography process steps of (1) contacting, (2) adsorbing, (3) eluting, and (4) cleaning in place for the reasons set forth in the Petition. Pet. 16–31; Ex. 1002 ¶ 24.

Linhult teaches making affinity chromatography columns with protein Z, Z(F30A), and other mutated variants. These modified proteins were covalently attached to HiTrap columns in Linhult using NHS-chemistry. Ex. 1004, 4. Linhult uses an affinity matrix column to isolate IgG and measures the loading capacity of the column after repeated cleaning in place (CIP) cycles. Ex. 1004, 4. In Linhult’s studies, human polyclonal IgG was prepared and injected onto the columns in excess and “[a] standard affinity chromatography protocol was followed.” *Id.* at 4. We find that Linhult’s loading of IgG onto the column satisfies the contacting step as recited in the claims. Ex. 1004, 4, *see also id.* (“The columns were pulsed with TST

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(25mM Tris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20) and 0.2 M HAc, pH 3.1. Human polyclonal IgG in TST was prepared and injected onto the columns in excess. A standard affinity chromatography protocol was followed for 16 cycles.”). Linhult teaches that “the amount of eluted IgG was measured after each cycle to determine the total capacity of the column.” *Id.* Linhult thereby expressly teaches the contacting and eluting steps, and following standard chromatography protocols the adsorbing step is implied. *Id.*; Ex. 1002 ¶ 24 (“After loading is completed, an additional step to wash out certain remaining impurities is employed. ([Ex. 1006 at 15–17]). Following the loading and wash steps, a different solution, typically one of low pH, is applied onto the column to elute the antibody”). Linhult also teaches a cleaning in place step using an alkaline cleaning agent. “The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min, resulting in a total exposure time of 7.5 h for Z(F30A) and mutants thereof.” Ex. 1004, 4.

Abrahmsén teaches using an IgG bound column for purifying the dimeric Z fragment from a supernatant. Ex. 1005, 9:60–10:15; Ex. 1002 ¶¶ 64–68. Abrahmsén affinity purification protocol is as follows:

The supernatant was passed through the column at a speed of 12 ml/h and the amount of IgG binding material [i.e. the Z fragment] was analyzed before and after it was run through the column. The bound material was washed with TS [(150 mM NaCl 50 mM tris HC pH 7.5)] supplemented with 0.05% Triton X-100 and then TS and finally with 0.05 M ammonium acetate before elution with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.

Ex. 1005, 10:8–16. The contacting step in Abrahmsén’s protocol occurs when the “supernatant was passed through the column,” the adsorbing step occurs before or during the time “[t]he bound material was washed,” and

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“elution” step occurs when the column is treated “with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.” *Id.* Although Abrahmsén column has IgG bound to the column, instead of an SPA domain, the reference still teaches the common chromatography steps of (1) contacting, (2) adsorbing, and (3) eluting a target molecule.

Hober teaches that “protein monomers can be combined-into multimeric proteins, such as dimers, trimers, tetramers, pentamers etc.”

Ex. 1006, 11. These monomer units can be linked with stretches of amino acids ranging from 0–15 amino acids. *Id.* Hober teaches

a matrix for affinity separation, which matrix comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which protein at least one asparagine residue has been mutated to an amino acid other than glutamine. . . The mutated protein ligand is preferably an Fc fragment-binding protein, and can be used for selective binding of IgG. . . the ligands present on the solid support comprising a multimer.

Ex. 1006, 13. Hober describes a typical chromatographic run cycle consisting of: sample application of 10 mg polyclonal human IgG; extensive washing-out of unbound proteins; elution at 1.0 ml/min with elution buffer; followed by Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0.5 M NaOH of 1 hour. *Id.* at 37.

Patent Owner does not dispute that the references disclose the recited chromatography process of contacting, adsorbing, eluting, and cleaning in place. *See generally* PO Resp.; *see id.* at 7 (citing Ex. 1002 ¶ 24; Ex. 2025 ¶ 43).

(b) Chromatography Matrix

The dispute between the parties is whether a person of ordinary skill in the art would have modified the process disclosed in Linhult using the

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G29A modified SPA C domain as disclosed in Abrahmsén. Pet. 22

(“Abrahmsén unequivocally discloses performing a G29A mutation on SPA’s C domain.”); *See* PO Resp. 17–54.

Linhult discloses a process for isolating one or more target compound(s) using chromatography matrices (solid support) comprising SPA ligands. Pet. 16–32. Linhult explains that SPA is a cell surface protein expressed by *Staphylococcus aureus* and consists of five highly homologous domains (E, D, A, B, and C). Ex. 1004, 1. Each of “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” *Id.* (bracketing in original). “Due to the high affinity and selectivity of SPA, it has a widespread use as an affinity ligand for capture and purification of antibodies.” Ex. 1004, Abstr., *see also id.* at 1 (“SPA has a widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.”).

Linhult explains that, in column chromatography, sodium hydroxide (NaOH) is probably the most extensively used cleaning agent for removing contaminants such as nucleic acids, lipids, proteins, and microbes, and a CIP step is often integrated in the protein purification protocols using chromatography columns. Ex. 1004, 1. “Unfortunately, protein-based affinity media show high fragility in this extremely harsh environment, making them less attractive in industrial-scale protein purification. SPA, however, is considered relatively stable in alkaline conditions.” *Id.* at 2. Linhult explains that the combination of asparagine with a succeeding glycine is the most sensitive amino acid sequence to alkaline conditions. *Id.* Linhult teaches that “[a]n exchange of glycine 29 for an alanine has been

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made in order to avoid the amino acid combination asparagine–glycine, which is [sensitive to alkaline conditions and is also] a cleavage site for hydroxylamine.” *Id.*

Petitioner’s declarant, Dr. Cramer avers that the “Z” domain referenced in Linhult refers to a synthetic version of the wild-type (i.e., natural) B domain of SPA, in which the naturally occurring glycine in the Asn₂₈-Gly₂₉ dipeptide sequence is replaced by an alanine residue to create an Asn₂₈-Ala₂₉ dipeptide sequence. Ex. 1002 ¶ 30 (citing Ex. 1004, 2, Fig. 1(a); Ex. 1007, 3, Fig. 1); ¶ 31 (citing Ex. 1005). We credit Petitioner’s declarant, Dr. Cramer for establishing that the C domain sequence disclosed in Linhult contains 55 amino acids in SEQ ID NO: 1 as claimed. Ex. 1002 ¶ 109 (showing a sequence alignment), *see also id.* ¶ 322 (showing sequence alignment of domain C of Abrahmsén with SEQ ID NO: 1).

According to Abrahmsén, the IgG binding domains E, D, A, B, and C of SPA were known. *See* Ex. 1005, 3:25–35, 4:34–37, Fig. 2. Relying on “computer analysis [Abrahmsén] surprisingly showed that the Gly in the Asn-Gly dipeptide sequence could be changed to an Ala. This change was not obvious as glycines are among the most conserved amino acids between homologous protein sequences due to their special features.” *Id.* at 5:7–9. Abrahmsén teaches that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. Thus, Abrahmsén provides motivation for making this mutation in any of the IgG binding domains E, D, A, B, and C of Staphylococcal protein A. Abrahmsén teaches recombinant DNA fragments coding for any of the E, D, A, B, and C domains of Staphylococcal protein A, wherein the glycine

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codon(s) in the Asn-Gly coding constellation have been replaced by an alanine codon. *Id.* at 2:33–37.

Abrahmsén, like Linhult, exemplifies the cloning of and expression of the Z-fragment. Ex. 1005, 7:65–10:56. Abrahmsén teaches that “the Z-region is the part of the Z-fragment coding for the IgG binding domain.” *Id.* at 3:39–41. Abrahmsén purifies the recombinant Z protein using an IgG column. *Id.* at 10:26–28. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén, therefore, reasonably suggests making multimeric constructs. Abrahmsén also uses column chromatography to purify a Z domain containing protein.

Hober teaches a multimer ligand that

also comprises one or more of the E, D, A, B, and C domains of *Staphylococcal* protein A. In this embodiment, it is preferred that asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids. In an embodiment advantageous for structural stability reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to, an alanine residue. Also, it is advantageous for the structural stability to avoid mutation of the asparagine residue in position 52, since it has been found to contribute to the α -helical secondary structure content of the protein A molecule.

Ex. 1006, 12, *see also id.* at 9 (“SEQ ID NO 1 defines the amino acid sequence of the B-domain of SpA”).

Here, the teachings of Linhult, Abrahmsén, and Hober suggest mutating the glycine at position 29 for an alanine in any one of the IgG binding domains of E D A B or C of SPA in order to avoid protein

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degradation. Ex. 1004, 2; Ex. 1005, 5:4–9. We, therefore, agree with Petitioner that the art expressly suggests that the glycine codon can be mutated for an alanine codon in any one of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111). Attaching any one of SPA mutated IgG binding domains E D A B or C to a matrix using “known ligand-construction methods to yield a predictable result[] (e.g., the claimed affinity chromatography matrix)” would have been obvious. Pet. 25 (citing Ex. 1002 ¶ 130). As the Federal Circuit has explained, “[w]here a skilled artisan merely pursues ‘known options’ from ‘a finite number of identified, predictable solutions,’ the resulting invention is obvious under Section 103.” *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421).

Accordingly, we agree with Petitioner that the combination of Linhult Abrahmsén, and Hober expressly suggests mutating the glycine codon for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 115–116). We also agree that the cited art teaches using these mutant SPA domains in column chromatography for the isolation of antibodies.

We address Patent Owner’s contentions below.

(2) *Response*

(a) *Matrix*

We do not find Patent Owner’s argument that the Petition fails to identify a reason to select domain C persuasive. PO Resp. 17–38. Specifically, we are not persuaded by Patent Owner’s contention that just because nobody was working on domain C at the time the invention was filed means selection of domain C would not have been obvious. *See* PO

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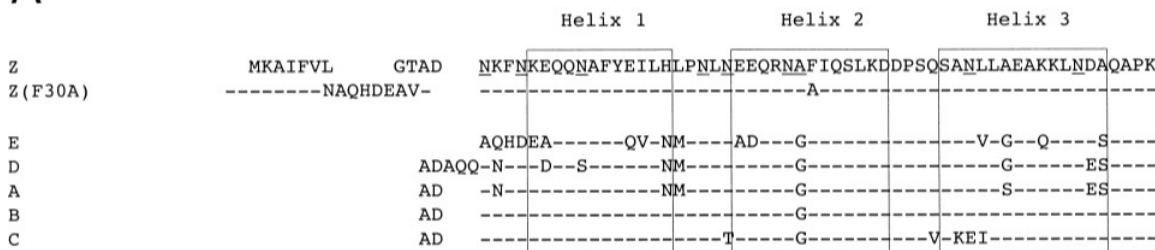
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Resp. 32 (“A general recognition that there exist five naturally occurring protein A domains is not a motivation to use each of them as a starting point for the claimed mutations”).

Petitioner’s articulated obviousness ground is premised on the knowledge that *any one* of the five SPA IgG binding domains are known to bind IgG and can function as a ligand for the purification of antibodies. Linhult and Abrahmsén both expressly suggest that the glycine codon at position 29 can be mutated for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Ex. 1004, 2; Ex. 1005, 2:32–37. Here, the SPA IgG binding domains comprise a short list of 5 members: E, D, A, B, or C. Of these 5 members, the glycine at position 29 in domain B has already been mutated to an alanine to create a domain Z which has been shown to retain IgG binding activity. Ex. 1004, 6 (Fig. 3). Figure 1A of Linhult is reproduced below.

A



Linhult’s Figure 1A, reproduced above, shows the amino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C). The three boxes show the α -helices. Ex. 1004, 2; Ex. 1005, Fig. 2.

Here, Linhult and Abrahmsén show that the IgG binding domains of SPA– E, D, A, B, or C share many structural similarities. *See* Ex. 1004, 2 (Fig. 1(a) (reproduced above)); Ex. 1005, 3:25–35. As discussed in our Institution Decision (Dec. 30–31), there are a finite number – five (5) – SPA

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IgG binding domains and each possesses the dipeptide sequence Asp-Gly known to be a target for alkaline protein degradation. Therefore, the solution of mutating the glycine at position 29 for an alanine to remove the alkaline sensitive sequence is not a product of innovation but of ordinary skill and common sense. *See Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421). It is well established that

[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties.

In re Deuel, 51 F.3d 1552, 1558 (Fed. Cir. 1995).

There is also an express teaching in both Linhult and Abrahmsén to mutate the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which further supports the obviousness of incorporating the mutation into any one of the IgG binding domains that has the Asn-Gly dipeptide. *See, e.g., SIBIA Neurosciences, Inc. v. Cadus Pharm. Corp.*, 225 F.3d 1349, 1358–59 (Fed. Cir. 2000) (stating that an express teaching in the prior art suggesting a particular modification establishes obviousness).

Because the G29A modification would have provided ligands that are less susceptible to alkaline conditions and are resistant to hydroxylamine cleavage, Petitioner has provided a sufficient evidence-backed reason for making the modification in any one of the domains. Pet. 16–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

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(b) Reasonable Expectation of Success

We are not persuaded by Patent Owner’s contention that there is no reasonable expectation of success in using a G29A mutation in domain C. PO Resp. 49–55.

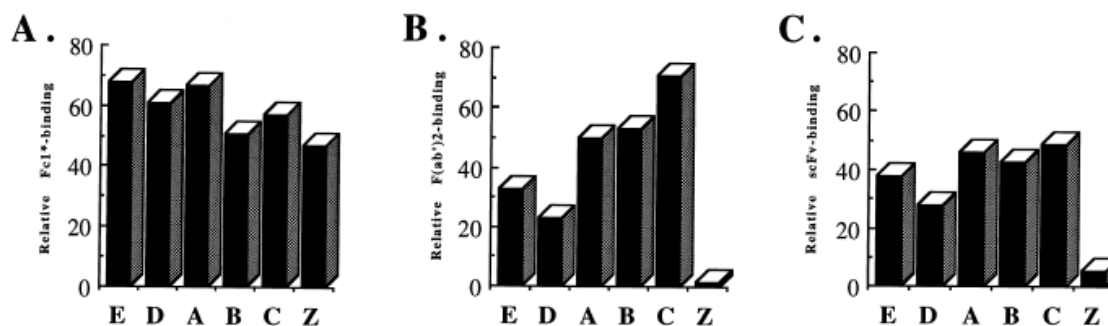
Linhult explains that removing the Asp–Gly amino acid combination not only results in the removal of the hydroxylamine cleavage site but also creates a product that is more alkaline resistant. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”).

Abrahmsén teaches that this Asn-Gly amino acid combination is present in all five SPA IgG binding domains and that mutating the dipeptide would not interfere with IgG binding. Ex. 1005, 4:56–58 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine. As this sequence is kept intact in all five IgG binding domains of protein A. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”). Abrahmsén’s conclusion that the mutation would not interfere with binding to IgG is supported by Abrahmsén (*see* Ex. 1005, 9:60–10:35), Linhult (*see* Ex. 1004, 6 (Fig. 3)), and Jansson (Ex. 2029).⁷ Jansson’s Fig. 3,

⁷ Patent Owner cites Jansson (Ex. 2029) for the position that domain Z has negligible binding to Fab. *See* PO Resp. 40–41. However, claims 1, 2, and 20 are not limited to Fab binding. Indeed the claims do not even require IgG binding.

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reproduced below.



Jansson Figure 3 (Panel A), reproduced above, shows a side-by-side comparison of Fc1*, Fab, and scFv binding to SPA domains. The figure shows that the single G29A mutation between domain B and domain Z results in a protein that is able to bind IgG.⁸ Comparing panel A - domain B domain with panel A - domain Z, the relative binding remains close to 50%, indicating that the G29A mutation between domains B and C does not interfere with IgG binding. Ex. 2029, 6. This is a result predicted by Abrahmsén's computer modeling and substantiated by Abrahmsén domain Z purification and Linhult's IgG purification. *See* Ex. 1005, 4:56–58, 9:60–10:35; Ex. 1004, 6 (Fig. 3).

“Obviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success.” *In re Droge*, 695 F.3d 1334, 1338 (Fed. Cir. 2012) (quoting *In re Kubin*, 561 F.3d 1351, 1360

⁸ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the “IgG control” in Jansson. Patent Owner's counsel explains that “Part A is Fc binding. So that is, I believe the way they did this experiment was with Fc fragments, but it's generally acknowledged, you know, these antibodies all have an Fc domain if they're a whole antibody and that reflects the fact that all of these domains A, B, C, D and E and domain Z, which is B with the G29A mutation, retain this Fc binding.” Tr. 70:6–11.

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(Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894, 903–04 (Fed. Cir. 1988)); *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016) (explaining that the expectation of success issue involves a showing of “a reasonable expectation of achieving *what is claimed*”) (emphasis added). “Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention.” *Pharma Stem Therapeutics, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1363–1364 (Fed. Cir. 2007).

Here, the record supports that each individual SPA domain, including the C domain, has affinity for IgG antibodies. Ex. 1004, 1 (“The five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” (bracketing in original)). Abrahmsén suggests making a mutation of Asn-Gly coding constellation in *any one* of the SPA domains by replacing a glycine codon with an alanine codon to remove the Asn-Gly dipeptide sequence known to be sensitive to hydroxylamine degradation. *See* Ex. 1005, 4:56–5:16, *see also id.* Fig. 2 (showing the Asn-Gly coding constellation in all SPA domains); Ex. 1006, 2 (“the shortest deamidation half times have been associated with the sequences –asparagine–glycine and – asparagine–serine”). Abrahmsén’s confirms that a G29A mutation on SPA would not interfere with folding of SPA protein and the binding to antibodies. Ex. 1005, 5:13–16 (“by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”), 9:60–10:35 (using IgG columns to purify protein Z dimers). Abrahmsén’s computer modeling suggests that IgG binding is not impacted

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by the mutation and this is confirmed by Linhult's experiments showing that the G29A mutant of domain B (a.k.a. domain Z) binds IgG. Ex. 1004, 6 (Fig. 3); *see also* Ex. 1005, 9:60–10:35.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

We are not persuaded by Patent Owner’s contention that the information gained by computer modeling of the SPA native domain B – IgG crystal structure could not be extrapolated to other SPA domains that are structurally very similar.

As Petitioner’s expert, Dr. Cramer explains

[i]t was well known that the researchers who developed the Z domain based on the wild-type B domain (rather than any of the other four SPA domains) did so for two reasons. (*See, e.g.*, Ex. 1007 at 109.) First, a crystal structure of the wild-type B domain binding to an antibody happened to be available in 1981 for analysis. (*See, e.g., id.*; Ex. 1005 at col. 4:56-68; Ex. 1017.) And, second, *their work would be informative of mutations that could be done on all five of the highly homologous SPA domains more generally.* (*See, e.g.*, Ex. 1005 at col. 2:32-37; Ex. 1007 at 109; Ex. 1008 at 639, Fig. 1.)

Ex. 1002 ¶ 33 (emphasis added). Dr. Cramer further explains that “[t]hey did the computer modeling based on that complex because that’s the crystal structure that they had. It wasn’t done because the B domain is special. . . . And then there’s several other places where they state clearly that they could also do the other domains with expected similar results.” Ex. 2015, 138:8–22.

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We agree with Petitioner that the teachings of Linhult, Abrahmsén, and Hober provide a reasonable expectation of success at arriving at a chromatography composition that contains the SPA domain C ligand with the G29A mutation that can be used in a process for purifying IgG. Pet. 24 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 124–126), *see id.* at 48–49 (citing Ex. 1006, 10–12; Ex. 1002 236–263).

(c) No Teaching Away

We are also not persuaded by Patent Owner’s contention that the art teaches away from the G29A substitution because it interferes with Fab binding. *See* PO Resp. 39–42; Sur-reply 9; Ex. 2009 at 2; Ex. 2010 at 25; Ex. 2012 at 25–26; Ex. 2029 at 7.

None of claims 1, 2, or 20 recite a need to bind the Fab region of an antibody or that the target molecule is Fab. All that is required by these claims is that they adsorb a target molecule and that you can elute the target molecule from the matrix. The target molecule, therefore, can reasonably encompass IgG.

Petitioner’s articulated rationale is that there was an expectation that the composition binds antibodies, including monoclonal antibodies, and therefore, would be useful in a process of isolating antibodies. Petitioner contends that:

A POSA would have also reasonably expected such a combination to achieve a process for isolating one or more target compounds using the recited affinity chromatography matrix given the well-known fact that each individual SPA domain, including the C domain, has affinity for antibodies (Ex. 1004, [1]) as well as *Abrahmsén*’s confirmation that a G29A mutation on SPA “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1005, 5:13-16). (Ex. 1002 ¶137.)

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Pet. 26; *see also* Reply 8 (“A POSA would not have been motivated only by Fab-binding ability, as even Dr. Bracewell agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’ (Ex. 1057, 75:17-76:4, 113:23-114:11, 157:24-158:9; Ex. 1061 ¶29)”).

The law does not require that the teachings of the reference be combined for the reason or advantage contemplated by the inventor, as long as some suggestion to combine the elements is provided by the prior art as a whole. *In re Beattie*, 974 F.2d 1309, 1312 (Fed. Cir. 1992); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976); *see In re Kemps*, 97 F.3d 1427, 1430 (Fed. Cir. 1996) (“[T]he motivation in the prior art to combine the references does not have to be identical to that of the applicant to establish obviousness.”).

Here, Linhult teaches that “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” Ex. 1004, 1 (bracketing in original) (citation omitted). Linhult, therefore, teaches that *any one* of the SPA IgG binding domains E, D, A, B, or C can bind the Fc region of an antibody and can therefore be used as a ligand for purifying IgG antibodies. In addition, the combination of Linhult and Abrahmsén suggests making the G29A mutation in each of the domains because it would provide ligands that are less susceptible to protein degradation. Ex. 1004, 2; *see also* Ex. 1005, 2:33-37 (“[A] recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.”).

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Patent Owner contends that the G29A mutation would lead to a reduction in the Fab binding of domain C, and therefore, would lead away from making the mutation. PO Resp. 40–41 (citing Ex. 2010, 2; Ex. 2011, 25; Ex. 2012, 25–26; Ex. 2013, 2–3; Ex. 2025 ¶¶ 105–109; Ex. 2029, 6–7). Patent Owner’s cited references are directed to Fab binding. But claims 1, 2, and 20 are not limited to Fab binding. Showing that the G29A mutation interferes with Fab binding does not teach away from mutating any of SPA domains E, D, A, B, or C in order to create an IgG binding ligand that retains its’ binding affinity for Fc yet is less sensitive to cleaning in place solutions. *See, e.g.*, Ex. 2013, 3 (“The site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ⁹ binding.”). Accordingly, we are not persuaded by Patent Owner’s contention that with respect to claims 1, 2, and 20 that the art teaches away from Petitioner’s proposed combination.

(d) Additional Modifications

We also disagree with Patent Owner’s contention that the ordinary artisan would not stop with a single G29A mutation in a SPA domain. *See* PO Resp. 44–48. Here, Abrahmsén expressly suggests making only a single mutation. Specifically, Abrahmsén contemplates “a recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation

⁹ Fcγ is the constant region of IgG involved in effector function. Specifically, “[t]he Fcγ binding site has been localized to the elbow region at the CH2 and CH3 interface of most IgG subclasses, and this binding property has been extensively used for the labeling and purification of antibodies.” Ex. 2013, 1. In other words, Fcγ and Fc terminology are used interchangeably in the art.

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has been replaced by an alanine codon” without additional mutations.

Ex. 1005, 2:33–37.

(3) *Summary*

We find that Petitioner has shown by a preponderance of the evidence that the combined teachings of at least Linhult and Abrahmsén suggests the use of *any one* of the SPA IgG binding domains E, D, A, B, or C as the starting ligand for purifying IgG antibodies, and that making the G29A mutation in *any one* of the domains would have been obvious because it would have provided ligands that are less susceptible to alkaline conditions and are resistant to hydroxylamine cleavage. Pet. 16–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

Having considered the evidence and argument cited in the Petition, which we have described above and find persuasive, we are persuaded that Petitioner has shown by a preponderance of evidence of record that the combination of Linhult, Abrahmsén, and Hober teaches each of the limitations of claims 1, 2, and 20. Petitioner not only has articulated a sufficient motivation for making the combination but has also established that there is a reasonable expectation of success for the binding of an IgG antibody to a SPA domain that contains an G29A mutation.

b) Claims 11 and 29

Petitioner argues that “[t]he ‘capab[ility] of binding to the Fab part of an antibody,’ as recited in claims 11 and 29, is an inherent property of the recited C(G29A)-based SPA ligand.” Pet. 30 (citing Ex. 1002 ¶¶ 155–162). Petitioner contends that a person of ordinary skill in the art did not need to recognize the Fab binding property of Domain C to be motivated to select

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that domain for modification. “A POSA would not have been motivated only by Fab-binding ability,[] as even Dr. Bracewell [Patent Owner’s expert] agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’” Reply 8 (citing Ex. 1057, 75:17–76:4, 113:23–114:11, 157:24–158:9; Ex. 1061 ¶ 29).

Patent Owner argues that

[t]he very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding “negligible” when implemented in Domain B. Ex. 2009 at 2; *see also, e.g.*, Ex. 2010 at 2 (“Fab binding activity is located to a region determined by helices 2-3, including the position mutated to yield the Z domain.”); Ex. 2011 at 25 (“[I]t only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine to an alanine”); Ex. 2012 at 25-26 (“[D]omain Z containing a single G29A-substitution compared to domain B exhibits little or no [Fab] binding. This might be due to the substitution since the C_β of the alanine would perturb the interaction between the two molecules.”).

PO Resp. 41.

Because claim 11¹⁰ is directed to a “[a] process for isolating one or more target compound(s)” and identifies that the target compound is “the Fab part of an antibody” (Ex. 1001, 16:66–67) the evidence needs to show a reasonable expectation that a mutated SPA ligand binds Fab. Without such a showing, there is no reasonable expectation that the process would result in the purification of a Fab target. In other words, because the claims are process claims the Petitioner needs to establish that a mutated SPA domain would reasonably bind a Fab fragment.

¹⁰ Claim 11 and 29 recite similar limitations.

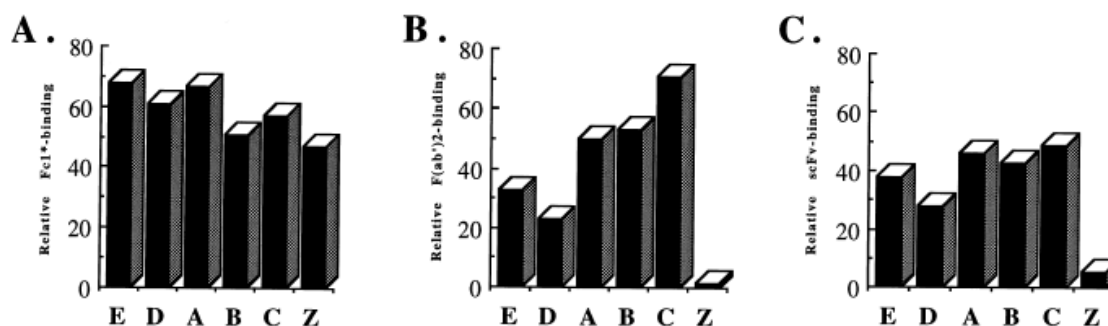
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We agree with Petitioner, that Linhult establishes that a G29A mutation in domain B (resulting in domain Z) does not interfere with IgG binding. *See* Ex. 1004, 6 (Fig. 3 (showing IgG binding with domain Z)). Linhult, however, is silent with respect to Domain Z's ability to bind to Fab fragments. *See generally* Ex. 1004. Abrahmsén similarly establishes Domain Z binding to IgG but is also silent with respect to Domain Z binding Fab. *See generally* Ex. 1005. Hober also does not disclose Fab binding of a mutant SPA domain. *See generally* Ex. 1006. Thus, each of Linhult, Abrahmsén, or Hober are silent with respect to Fab binding to a mutated SPA domain.

Jansson (Ex. 2029), cited by Patent Owner, supports the position that Fab binding to mutated SPA domains is unpredictable. Jansson, just like Linhult, recognizes that “[a]ll [SPA] domains bound to a recombinant human IgG1 Fc fragment with similar strength. For the first time, binding to human Fab was demonstrated for all *native SPA domains*, using both polyclonal F(ab')₂ and a recombinant scFv fragment as reagents.” Ex. 2029, Abstract (emphasis added). Jansson, however, establishes that “the engineered Z domain showed a considerably lower affinity for Fab as compared to the native domains.” *Id.* Jansson Fig. 3, reproduced below, shows that the G29A mutation results in a loss of Fab binding ability.



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Jansson Figure 3, reproduced above, shows the side-by-side comparison of Fc1*¹¹, Fab, and scFv binding and confirms what was already suggested in Linhult, Abrahmsén, and Hober – that a composition containing the G29A mutation in a SPA domain can bind IgG. Ex. 2029, 6 (Fig. 3 (*compare* Panel A-domain B, *with* Panel A-domain Z). Panel B in Jansson Figure 3, however, shows that the single G29A mutation between Domain B and Domain Z results in the loss of Fab binding. Ex. 2029, 6 (Fig. 3 (*compare* Panel B-Domain B, *with* Panel B-Domain Z). At the time the invention was made it was also known that “[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding.” Ex. 2013, 3. Thus, on this record, establishing that a mutation that does not interfere with IgG binding says nothing about the ability of a mutated SPA domain to bind Fab.

We, therefore, agree with Patent Owner’s contention that based on the prior art, the Fab binding capacity was unknown with the modification as suggest by the combination of Linhult, Abrahmsén, and Hober.¹² Patent Owner has provided evidence that Fab biding capacity of a mutated SPA

¹¹ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the IgG control in Jansson. Fc1* is functionally equivalent to Fcγ in SPA binding.

¹² In *JSR Corporation et al. v. Cytiva Bioprocess R&D AB et al.*, IPR2022-00036, Paper 41 at 44–49 (PTAB April 19) (Final Written Decision) we determined that the capability of SEQ ID NO:1 to bind Fab is an inherent feature of the structure claimed. The present claims, however, are directed to a method of isolating Fab which requires prior knowledge that the ligand binds Fab. Fab is a digestion product of a whole IgG molecule treated with papain and is not naturally found in IgG samples. *See above* I.F. In other words, when isolating IgG with a column containing SEQ ID NO: 1 there would be no elution of Fab because the fragments are not present in an IgG containing sample.

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domain protein is unpredictable. *See* PO Resp. 56–57 (Ex. 2029, 5–6). The disclosures in the prior art, therefore, support Patent Owner’s position that “the SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” PO Resp. 57 (citing Ex. 2025 ¶ 123; Ex. 2030, 18–19).

Because the art does not support the conclusion that G29A mutation in a SPA domain ligand binds Fab, Petitioner has not established by a preponderance of the evidence of record that the process of isolating Fab target using a mutated SPA domain C ligand as required by claims 11 and 29 would have been obvious based on the combined teachings of Linhult, Abrahmsén, and Hober.

c) Claims 3–8 and 21–26

Claims 3–8 depend either directly or indirectly from claim 1, and claims 21–26 depend either directly or indirectly from claim 20. These claims further limit the recited processes of claims 1 and 20 whereby the recited matrix retains a percentage of its original binding capacity after certain NaOH exposures.

We are not persuaded by Patent Owner’s argument that “none of Petitioners’ cited references actually describe a C(G29A)-based SPA ligand, let alone provide alkaline stability data or test results concerning the same, the POSA is simply left to guess at how such a ligand would perform.” PO Resp. 50 (citing Ex. 2025 ¶¶ 121–124).

For the reasons discussed above (II.E.5.a), we find that Petitioner has shown by a preponderance of evidence that there is a reason to make the G29A mutation in any one of the SPA IgG binding domains, including

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domain C, and that the use of the mutated protein would reasonably result in a matrix that can be used to purify IgG. Linhult teaches a CIP protocol with at 0.1 to 0.5M NaOH was a well-known and conventional technique. Pet. 30 (citing Ex. 1004, 1–2, 4–5; Ex. 1002 ¶ 152); Ex. 1004, 6 (“Figure 3, the Z(N23T) mutant shows higher resistance to alkaline conditions than the Z domain when exposed to high pH values.”); *see also* Ex. 1006, 39 (“Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0,5 M NaOH of 1 hour”). “Between each cycle [in Linhult], a CIP-step was integrated. The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min.” Ex. 1004, 4. “After 16 [CIP] cycles, giving a total exposure time of 7.5 h, the column with the Z(F30A)-matrix shows a 70% decrease in capacity.” Ex. 1004, 5; *see also* Ex. 1006, 39 (“Each cycle [in Hober] was repeated 21 times resulting in a total exposure time between the matrix and the sodium hydroxide of 20 hours for each different matrix”). Both Linhult and Hober recognize that repeated exposures of a SPA chromatography ligand leads to a reduction of the binding capacity over time. That Linhult recognizes that additional mutations could further improve alkaline stability does not detract from Linhult’s teaching that a composition containing the single G29A mutation in SPA domain B retains IgG binding. Ex. 1004, 6, *see id.* at 4 (“The Z-domain already possesses a significant tolerance to alkaline conditions.”).

Petitioner has shown by a preponderance of the evidence of record that there is a reason for making the G29A mutation in *any one* of the four remaining SPA domains in order to produce a SPA product that is more alkaline stable and would reasonably bind IgG. *See* Pet. 26–28, 30; Reply 20–21; *see* Ex. 1002 ¶¶ 115–119.

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d) Claims 9, 10, 12–14, 16–19, 27–28, 30–32 and 34–37

Petitioner asserts that Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims. *See* Pet. 26–30, 37–41. In support of these arguments, Petitioner directs our attention to the relevant disclosures of Linhult, Abrahmsén, and Hober and provides a detailed claim analysis addressing how each element of the challenged claims are disclosed by the cited prior art and provides explanation as to why one of ordinary skill in the art would have combined the references to arrive at the claimed subject matter with a reasonable expectation of success. *Id.*

Patent Owner does not offer arguments addressing Petitioner’s substantive showing with respect to claims 9, 10, 12–14, 16–19, 27–28, 30–32 and 34–37 separate from its arguments about claims 1, 2, and 20. *See generally* PO Resp.

We have reviewed Petitioner’s arguments and the underlying evidence cited in support, which we adopt as our own, and determine that Petitioner establishes that the combination of Linhult, Abrahmsén, and Hober teaches the additional limitations of these dependent claims. *See e.g.*, Pet. 26 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 112–114), *see id.* at 30 (citing Ex. 1002 ¶¶ 128–129); Ex. 1004, 4 (“a multimerization of the domain to achieve a protein A-like molecule”); Ex. 1005, 9:15–10:35. In particular, we note that Hober teaches that monomeric mutant proteins can be combined into multimeric proteins, such as dimers, trimers, tetramers, pentamers, and other multimers. Ex. 1006, 11. Hober also discloses that the multimer comprises mutant monomer units “linked by a stretch of amino acids preferably ranging from 0 to 15 amino acids, such as 5-10.” *Id.* Petitioner also provides sufficient explanation as to why one of ordinary skill in the art would have combined

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the references to arrive at the claimed subject matter with a reasonable expectation of success. Pet. 26–30, 37–41.

Accordingly, we determine that the preponderance of evidence of record supports Petitioner’s contentions with respect to claims 9, 10, 12–14, 16–19, 27–28, 30–32 and 34–37.

6. Summary

For the foregoing reasons, we determine that Petitioner has shown by a preponderance of evidence that of claims 1–10, 12–14, 16–28, 30–32, and 34–37 of the ’007 patent are unpatentable based on the combination of Linhult, Abrahmsén, and Hober.

For the reasons discussed above, Petitioner has not shown by a preponderance of evidence that of claims 11 and 29 of the ’007 patent are unpatentable.

F. Other Asserted Grounds

1. The ’042 IPR Grounds Based on Other Various Combinations of Linhult, Abrahmsén, and Hober

Petitioner also asserts that claims 1–11 and 20–29 are unpatentable as obvious over Linhult and Abrahmsén (Pet. 18–30); that claims 1–14, 16–32, and 34–37 are unpatentable as obvious over Linhult and Hober (*id.* at 30–48); and that claims 1–14, 16–32, and 34–37 are unpatentable as obvious over Abrahmsén and Hober (*id.* at 49–60) under 35 U.S.C. §103(a).

Because Petitioner has already shown that challenged claims 1–10, 12–14, 16–28, 30–32, and 34–37 are unpatentable over Linhult, Abrahmsén, and Hober as obvious, as discussed *supra*, we do not reach these claims in these additional asserted grounds as to those claims. *See Beloit Corp. v.*

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Valmet Oy, 742 F.2d 1421, 1423 (Fed. Cir. 1984) (“The Commission . . . is at perfect liberty to reach a ‘no violation’ determination on a single dispositive issue.”); *Boston Sci. Scimed, Inc. v. Cook Grp., Inc.*, 809 F. App’x 984, 990 (Fed. Cir. 2020) (recognizing that “[t]he Board has the discretion to decline to decide additional instituted grounds once the petitioner has prevailed on all its challenged claims”).

Petitioner has not shown that the challenged claims 11 and 29 are unpatentable over Linhult, Abrahmsén, and Hober. We note that each of Linhult, Abrahmsén, and Hober is silent with respect to Fab binding to a mutant SPA domain. *See above* II.E.5.b. Patent Owner asserts, and we agree, that there is no reasonable expectation that a G29A SPA domain mutant would bind Fab. Specifically, Patent Owner’s cited references show that a G29A mutation in domain B results in a domain Z matrix composition that *does not* bind Fab. *See* Ex. 2029, 6 (Fig. 3 (*compare* Panel B-domain B, *with* Panel B-domain Z); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”). For the reason discussed above (II.E.5.b), Petitioner has not shown by a preponderance of the evidence of record that a G29A SPA domain mutant would bind a Fab fragment so that a process of using the G29A SPA domain ligand would reasonably result in the purification of the Fab target. That deficiency in Petitioner’s showing persists whether the grounds are based on the combination of Linhult, Abrahmsen, and Hober (discussed above) or the related combinations of Linhult combined with Abrahmsen or Hober or Abrahmsen combined with Hober.

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2. *The '045 IPR Grounds Based on Various Combinations of Berg¹³, Linhult, Abrahmsén, and Hober*

Petitioner asserts that claims 1–14, 16–18, 20–32, and 34–36 are unpatentable as obvious over Berg¹⁴ and Linhult ('045 IPR Pet. 21–39); claims 4–8, 10, 19, 22–26, 28, and 37 are unpatentable as obvious over Berg, Linhult, and Hober (*id.* at 39–46); that claims 1–3, 9, 10, 12–14, 16–18, 20, 21, 27, 28, 30–32, and 34–36 are unpatentable as obvious over Berg and Abrahmsén (*id.* at 47–54); and that claims 4–8, 10, 11, 19, 22, 26, 28, 29, and 37 are unpatentable as obvious over Berg, Abrahmsén, and Hober (*id.* at 54–57) under 35 U.S.C. §103(a).

Berg relates to a chromatography matrix to which antibody-binding protein ligands are immobilized. Ex. 1018, Abstract. Petitioner relies on a single paragraph in Berg, paragraph 29, for teaching antibody binding ligands including SPA domain C. *See* '045 IPR Pet. 24–25, 48. The remainder of the Berg reference is directed to the structure of the chromatography matrix. *See generally* Ex. 1018. A review of Berg shows that SPA is mentioned at three locations in the reference. *See* Ex. 1018 ¶¶ 28, 29, and claim 12. Paragraph 29 of Berg suggests using ligands made up of one or more domains A, B, C, D, and E, and preferably domain B and/or domain C. Ex. 1018 ¶ 29. Just like Linhult, Abrahmsén, and Hober, Berg

¹³ Berg et al., US 2006/0134805 A1, published June 22, 2006. Ex. 1018.

¹⁴ We recognize that there is a dispute between the parties whether Berg qualifies as a 35 U.S.C. §102(a) date reference or a §102(b) date reference. *See* Pet; PO Resp., Reply, and Sur-reply. Because we do not need to reach these additional asserted grounds based on Berg beyond addressing whether Berg teaches Fab binding in the context of a mutant SPA domain ligand to establish that Berg does not address the deficiency of the Linhult, Abrahmsén, and Hober combination, we, therefore, do not need to address the prior art status of Berg.

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also does not say anything about the ability of a mutant SPA domain ligand, including a domain C ligand, to bind Fab.

Patent Owner, however, cites prior art references to establish that at the time the invention was made there was no expectation that a G29A SPA domain mutant would bind Fab. *See above* II.E.5.b; *see* '045 IPR PO Resp. 43, 54, 55, 58. Specifically, Patent Owner's cited references showing that a G29A mutation in domain B results in a domain Z matrix composition that does not bind Fab. *See id.*; Ex. 2029, 6 (Fig. 3 (compare Panel B-domain B, with Panel B-domain Z); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”).

Berg, therefore, does not address Fab binding in the context of a mutant SPA domain ligand, specifically domain C, nor does Berg explain why one of ordinary skill in the art would have reasonably expected domain C to retain the ability to bind Fab when other SPA domain mutants do not retain this feature. Because Berg does not address the deficiency of Linhult, Abrahmsén, and Hober as identified by Patent Owner and discussed above (II.E.5.b), any combination of Berg in conjunction with Linhult, Abrahmsén, and/or Hober would not address the missing limitation of claims 11 and 29. Therefore, we do not reach these additional asserted grounds based on Berg beyond addressing whether Berg addresses this missing limitation. *See Beloit Corp.*, 742 F.2d at 1423.

III. CONCLUSION¹⁵

For the foregoing reasons, we determine that Petitioner has demonstrated by a preponderance of the evidence that claims 1–10, 12–14,

¹⁵ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this

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16–28, 30–32, and 34–37 of the '007 patent are unpatentable, and that claims 11 and 29 have not been shown unpatentable on the bases set forth in the following table.

In summary:

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–11, 20–29	103(a)	Linhult, Abrahmsén ¹⁶		11, 29
1–14, 16–32, 34–37	103(a)	Linhult, Hober ¹⁷		11, 29
1–14, 16–32, 34–37	103(a)	Linhult, Abrahmsén, Hober	1–10, 12–14, 16–28, 30–32, 34–37	11, 29
1–14, 16–32, 34–37	103(a)	Abrahmsén, Hober ¹⁸		11, 29

decision, we draw Patent Owner's attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. See 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. See 37 C.F.R. § 42.8(a)(3), (b)(2).

¹⁶ As explained above (II.F.1), we do not reach claims 1–10 and 20–28 in this '042 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these claims.

¹⁷ As explained above (II.F.1), we do not reach claims 1–10, 12–14, 16–28, 30–32, and 34–37 in this '042 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

¹⁸ As explained above (II.F.1), we do not reach claims 1–10, 12–14, 16–28, 30–32, and 34–37 in this '042 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged *claims*.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
Overall Outcome			1–10, 12–14, 16–28, 30–32, 34–37	11, 29

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–14, 16– 18, 20–32, 34–36	103(a)	Berg, Linhult ¹⁹		11, 29
4–8, 10, 19, 22–26, 28, 37	103(a)	Berg, Linhult, Hober ²⁰		
1–3, 9, 10, 12–14, 16– 18, 20, 21, 27, 28, 30– 32, 34–36	103(a)	Berg, Abrahmsén ²¹		

¹⁹ As explained above (II.F.2), we do not reach claims 1–10, 12–14, 16–18, 20–28, 30–32, and 34–36 in this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

²⁰ As explained above (II.F.2), we do not reach this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²¹ As explained above (II.F.2), we do not reach this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
4–8, 10, 11, 19, 22, 26, 28, 29, 37	103(a)	Berg, Abrahmsén, Hober ²²		11, 29
Overall Outcome			1–10, 12–14, 16–28, 30–32, 34–37	11, 29

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the preponderance of the evidence of record has shown that claims 1–10, 12–14, 16–28, 30–32, and 34–37 of the '007 patent are found unpatentable;

ORDERED that the preponderance of the evidence of record has not shown that claims 11 and 29 of the '007 patent are found unpatentable; and

FURTHER ORDERED because this is a final written decision, the parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

²² As explained above (II.F.2), we do not reach claims 4–8, 10, 19, 22, 26, 28, 37 in this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

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Paper 44
Date: April 19, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

JSR CORPORATION and JSR LIFE SCIENCES, LLC,
Petitioner,

v.

CYTIVA BIOPROCESS R&D AB,
Patent Owner.

IPR2022-00036
IPR2022-00043
Patent 10,213,765 B2

Before ULRIKE W. JENKS, SHERIDAN K. SNEDDEN, and
SUSAN L. C. MITCHELL, *Administrative Patent Judges*.

JENKS, *Administrative Patent Judge*.

JUDGMENT
Consolidated Final Written Decision
Determining All Challenged Claims Unpatentable
35 U.S.C. § 318

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I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review of claims 1–7, 10–20, and 23–26 (“the challenged claims”) of U.S. Patent No. 10,213,765 B2 (Ex. 1001, “the ’765 patent”). We have jurisdiction under 35 U.S.C. § 6, and enter this Consolidated Final Written Decision pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons set forth below, we determine that JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) has shown, by a preponderance of the evidence, that the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e).

A. Consolidated Proceedings

The two captioned proceedings (IPR2022-00036 and IPR2022-00043 (or “the ’043 IPR”) involve the ’765 patent and challenge the same set of claims. The asserted grounds and prior art contentions are different in each proceeding. Consolidation is appropriate where, as here, the Board can more efficiently handle the common issues and evidence, and also remain consistent across proceedings. Under 35 U.S.C. § 315(d), the Director may determine the manner in which these pending proceedings may proceed, including “providing for stay, transfer, consolidation, or termination of any such matter or proceeding.” *See also* 37 C.F.R. § 42.4(a) (“The Board institutes the trial on behalf of the Director.”). There is no specific Board Rule that governs consolidation of cases. But 37 C.F.R. § 42.5(a) allows the Board to determine a proper course of conduct in a proceeding for any situation not specifically covered by the rules and to enter non-final orders to administer the proceeding. Therefore, on behalf of the Director under § 315(d), and for a more efficient administration of these proceedings, we

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consolidate IPR2022-00036 and IPR2022-00043 for purposes of rendering this Final Written Decision.

B. *Procedural History*

JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) filed a Petition for an *inter partes* review of claims the challenged claims under 35 U.S.C. § 311 in each proceeding. Paper 1¹ (“Pet.”). Petitioner supported the Petition with the Declaration of Dr. Steven M. Cramer. Ex. 1002. Cytiva Bioprocess R&D AB (“Patent Owner”) filed a Patent Owner Preliminary Response to the Petition. Paper 9 (“Prelim. Resp.”).

On April 21, 2022, pursuant to 35 U.S.C. § 314(a), we instituted trial (“Decision” or “Dec.” (Paper 10)) to determine whether any challenged claim of the ’765 patent is unpatentable.

In IPR2022-00036, Petitioner asserts the following grounds of unpatentability (Pet. 4):

Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–4, 12, 14–17, 25	103(a)	Linhult, Abrahmsén
1–7, 10–20, 23–26	103(a)	Linhult, Hober

¹ We note that the evidence filed in both proceedings is generally consistent in having the same exhibit number. Therefore, we reference exhibits and paper numbers as they appear in the record of IPR2022-00036, unless otherwise noted.

² The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the ’765 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–7, 10–20, 23–26	103(a)	Linhult, Abrahmsén, Hober
1–7, 10–20, 23–26	103(a)	Abrahmsén, Hober

In IPR2022-00043, Petitioner asserts the following grounds of unpatentability ('043 IPR Pet. 4):

Claim(s) Challenged	35 U.S.C. §³	Reference(s)/Basis
1-7, 10–20, 23–26	103(a)	Berg, Linhult
2, 3, 15, 16	103(a)	Berg, Linhult, Hober
1, 2, 5–7, 10–15, 18–20, 23–26	103(a)	Berg, Abrahmsén
2–4, 15–17	103(a)	Berg, Abrahmsén, Hober

Patent Owner filed a Patent Owner Response to the Petition. Paper 16 (“PO Resp.”). Patent Owner supported the Response with the Declaration of Dr. Daniel Bracewell (Ex. 2025). *See* PO Resp., iv (Exhibit List). Petitioner filed a Reply to the Patent Owner Response. Paper 29 (“Reply”). Petitioner supported the Reply with a Reply Declaration from Dr. Steven M. Cramer. Ex. 1061. Patent Owner filed a Sur-reply to Petitioner’s Reply. Paper 35 (“Sur-reply”).

³ The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the ’765 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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On February 16, 2023, the parties presented arguments at an oral hearing. Paper 36. The hearing transcript has been entered in the record. Paper 40 (“Tr.”).

For the reasons set forth below, we determine that Petitioner has shown by a preponderance of the evidence that claims 1–7, 10–20, and 23–26 of the ’765 patent are unpatentable.

C. *Real Parties in Interest*

Petitioner identifies itself, JSR Corporation and JSR Life Sciences, LLC, along with JSR Micro NV, as the real parties-in-interest. Pet. 2. Patent Owner identifies itself, Cytiva Bioprocess R&D AB, along with Cytiva Sweden AB and Danaher Corporation as real parties-in-interest. Paper 7, 1.

D. *Related Matters*

The ’765 patent is at issue in *Cytiva BioProcess R&D et al. v. JSR Corp. et al.*, Civil Action No. 1:21-cv-00310 (D. Del.). Pet. 2; Paper 5, 1.

In addition to the ’765 patent challenged here, Petitioner has filed Petitions for *inter partes* review of related U.S. patents as follows: U.S. Patent No. 10,343,142 B2 (“the ’142 patent”) in IPR2022-00041 and IPR2022-00044; and U.S. Patent No. 10,875,007 B2 (“the ’007 patent”) in IPR2022-00042 and IPR2022 00045. Pet. 2–3; Paper 7, 1–2. Petitioner indicates that the ’142 patent and the ’007 patent are also being asserted in the above-cited district court case. Pet. 3. The parties further list a pending application in the same family, U.S. App. Serial No. 17/107,600. Pet. 2; Paper 7, 2.

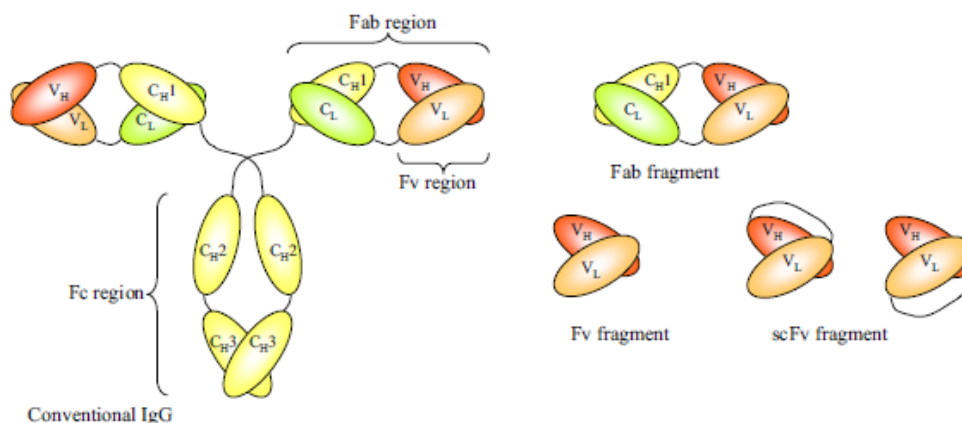
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E. *Subject matter background*

Antibodies (also called immunoglobulins) are glycoproteins, which specifically recognize foreign molecules. These recognized foreign molecules are called antigens. Ex. 2001, 1. A schematic representation of the structure of a conventional IgG and fragments is shown below:



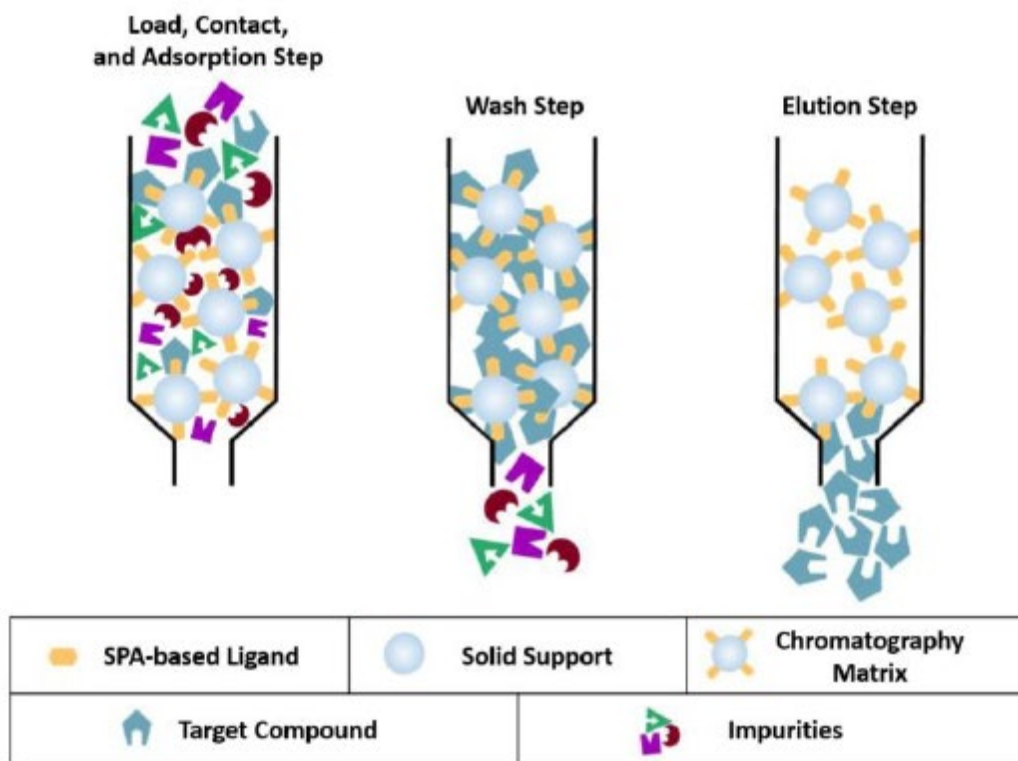
The figure (Ex. 2001, 2 (Fig. 1)), reproduced above, shows

the structure of a conventional IgG and fragments that can be generated thereof. The constant heavy-chain domains CH1, CH2 and CH3 are shown in yellow, the constant light-chain domain (CL) in green and the variable heavy-chain (VH) or light-chain (VL) domains in red and orange, respectively. The antigen binding domains of a conventional antibody are Fabs and Fv fragments. Fab fragments can be generated by papain digestion. Fvs are the smallest fragments with an intact antigen-binding domain. They can be generated by enzymatic approaches or expression of the relevant gene fragments (the recombinant version). In the recombinant single-chain Fv fragment, the variable domains are joined by a peptide linker. Both possible configurations of the variable domains are shown, i.e. the carboxyl terminus of VH fused to the N-terminus of VL and vice versa.

Ex. 2001, 2; *see also* PO Resp. 5.

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Below is a generic, exemplary schematic that shows how affinity purification typically works:



The figure shows the schematic of the loading, contact, and adsorbing step onto a column, followed by the wash step, and finally the elution and collection of the target compound. Ex. 1002 ¶ 24 (citing Ex. 1014 at §§ 1.1, 4.2.); *see also* PO Resp. 7 (“In a typical process, the composition containing the desired antibody then is loaded onto (i.e., pumped or injected into) the column.”); Pet. 6; *see generally* Ex. 1014.

F. *The '765 patent (Ex. 1001)*

The '765 patent is titled “Chromatography Ligand Comprising Domain C from *Staphylococcus Aureus* Protein A for Antibody Isolation.” Ex. 1001, (54). The '765 patent relates to an affinity ligand that is used for antibody isolation. *Id.* at 1:39–41. The '765 patent explains that

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chromatography is used in large-scale economic production of drugs and diagnostics in which proteins are produced by cell culture and then separated from the mixture of compounds and other cellular components to a sufficient purity. *Id.* at 1:52–61. One type of chromatography matrix for this purifying process includes immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG). *Id.* at 2:4–13. The '765 patent further explains that “[a]s in all process technology, an important aim is to keep the production costs low” by reusing matrices via cleaning protocols such as an alkaline protocol known as cleaning in place (CIP). *Id.* at 2:14–29. However, harsh treatments may impair the chromatography matrix materials such that there is a need for stability towards alkaline conditions for an engineered protein ligand. *Id.* at 2:31–48.

The '765 patent discloses that Protein A, known as SPA, is a constituent of the cell wall of the bacterium *Staphylococcus aureus*, and is widely used as a ligand in affinity chromatography matrices due to its ability to bind with IgG. *Id.* at 2:49–54. SPA is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind to antibodies at the Fc region, and it has been shown that each of these domains binds to certain antibodies at the Fab region. *Id.* at 2:54–63.

Domain C from SPA is defined by SEQ ID NO: 1 and is reproduced below.

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Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1           5           10           15
Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
20           25           30
Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
35           40           45
Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50           55

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Id. at 4:27; 15:1–20. SEQ ID NO: 1 shows Domain C has Glycine (Gly) as an amino acid at the position 29 as annotated via red highlighting. According to the '765 patent, it has already been shown “that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A” and the '765 patent discloses that from experiments, “the present inventors have quite surprisingly shown that the SPA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product.”

Id. at 5:38–40, 51–55. The '765 patent discloses, “it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences -asparagine-glycine- and -asparagine-serine.” *Id.* at 5:62–66. The '765 patent then discloses “[q]uite surprisingly, the Domain C ligand of the invention presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29” and “[t]hus, in a specific embodiment, the chromatography ligand according to the invention comprises SPA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A.” *Id.* at 5:67–6:3, 6:49–52. The '765 patent discloses that a multimeric chromatography ligand (also denoted a “multimer”) can be comprised of at least two Domain C units and that a

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chromatography matrix can be comprised of ligands coupled to an insoluble carrier. *Id.* at 7:27–29, 8:21–23. The '765 patent discloses a column study of alkaline stability of its Protein A-derived ligands and a testing of the Fab binding of its ligands. *Id.* at 10:32–14:59 (Example 1). The study includes using an injection liquid and solution along with human normal immunoglobulin as a target compound in chromatography experiments, ligand coupling and column packing, adsorbance measurements, washing out unbound samples, and eluting bound material. *Id.* at 11:11–18, 12:25–34, 13:32–37.

1. Illustrative Claim

Claims 1 and 14 are the independent claims challenged by Petitioner in this proceeding. Independent claim 1, reproduced below, is illustrative of the subject matter:

1. A chromatography matrix comprising:

a solid support; and a ligand coupled to the solid support, the ligand comprising at least two polypeptides,

wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and

wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.

Ex. 1001, 15:39–48. Claim 14 is similar to claim 1 but recites “at least 55 amino acids in alignment with SEQ ID NO. 1” rather than “at least 55 contiguous amino acids of a modified SEQ ID NO. 1,” and wherein “each polypeptide has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1” rather than “the modified

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SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.” *Id.* at 16:51–60.

II. ANALYSIS

A. *Principles of Law*

“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify “with particularity . . . the evidence that supports the grounds for the challenge to each claim”)). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner must demonstrate by a preponderance of the evidence⁴ that the claims are unpatentable. 35 U.S.C. § 316(e); 37 C.F.R. § 42.1(d). A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time of the invention to a person having ordinary skill in the art. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations, including “the scope and content of the prior art”; “differences between the prior art and the claims at issue”; “the

⁴ The burden of showing something by a preponderance of the evidence requires the trier of fact to believe that the existence of a fact is more probable than its nonexistence before the trier of fact may find in favor of the party who carries the burden. *Concrete Pipe & Prods. of Cal., Inc. v. Constr. Laborers Pension Tr. for S. Cal.*, 508 U.S. 602, 622 (1993).

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level of ordinary skill in the art;” and “objective evidence of non-obviousness.” *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. *Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the “type of problems encountered in the art, [the] prior art solutions to those problems, [the] rapidity with which innovations are made, [the] sophistication of the technology, and [the] educational level of active workers in the field.” *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

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Petitioner asserts that a person of ordinary skill in the art would have had

(1) at least an advanced degree (*e.g.*, a Master’s or Ph.D.) in biochemistry, process chemistry, protein chemistry, chemical engineering, molecular and structural biology, biochemical engineering, or similar disciplines; (2) several years of post-graduate training or related experience (including industry experience) in one or more of these areas; and (3) an understanding of the various factors involved in purifying proteins using chromatography.[] Such a person would have had multiple years of experience with affinity ligand design and protein purification.

Pet. 10–11 (citing Ex. 1002 ¶¶ 13–14). Patent Owner does not dispute Petitioner’s definition of the person of ordinary skill. *See generally* PO Resp. Because Petitioner’s proposed definition is unopposed and appears consistent with the Specification and art of record, we apply it here.

C. *Claim Construction*

The Board applies the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.200(b) (2021). Under that standard, claim terms “are generally given their ordinary and customary meaning” as understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc).

Petitioner argues that based on Patent Owner’s implicit construction in the district court litigation “the term ‘the ligand comprising at least two polypeptides’ refers to a multimeric ligand (such as a tetramer) comprised of multiple polypeptides, each of which is a monomer.” Pet. 17 (citing Ex. 1020 ¶¶ 41, 50, 58, 62, 74, 87).

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Patent Owner does not contest Petitioner’s construction. *See generally* PO Resp.

According to the Specification, “a multimeric chromatography ligand (also denoted a ‘multimer’) comprised of at least two Domain C units, or a functional fragments or variants thereof.” Ex. 1001, 7:27–30. The Specification additionally recites that a multimer containing only Domain C units can, however, include linkers. *Id.* at 7:39–41. In addition, the Specification describes that “the multimer comprises one or more additional units, which are different from Domain C.” *Id.* at 7:44–45. Based on these disclosures in the Specification, a multimer is composed of at least two or more monomers.

Because Petitioner’s construction is consistent with the ’765 patent’s express construction of the term, and because Patent Owner does not contest Petitioner’s construction, we apply it here.

D. *Overview of Asserted References*

1. *Linhult (Ex. 1004)*

Linhult is titled “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach.” Ex. 1004, 1. Linhult discloses that due to the high affinity and selectivity of Staphylococcal protein A (SPA), “it has a widespread use as an affinity ligand for capture and purification of antibodies” but that “it is desirable to further improve the stability in order to enable an SPA-based affinity medium to withstand even longer exposure to the harsh conditions associated with cleaning-in-place (CIP) procedures.” *Id.*, Abst. Linhult discloses, “[t]o further increase the alkaline tolerance of SPA, we chose to

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work with Z, which is a small protein derived from the B domain of SPA.”
Id. at 2.

Figures 1A and 1B of Linhult are reproduced below.

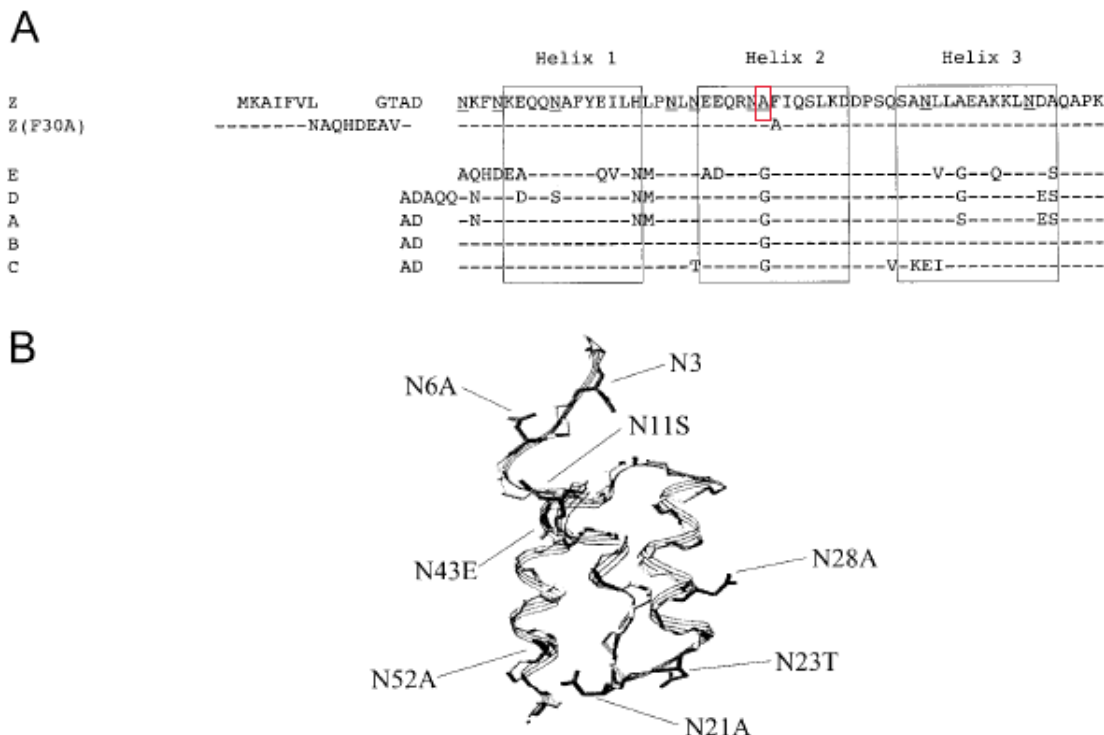


Figure 1A shows “[a]mino acid alignments of the Z, Z(F30A) and the five [naturally occurring] homologous domains (E, D, A, B, and C)” in which the horizontal lines indicate amino acid identity and “one glycine in the B domain [is] replaced [and] underlined” as annotated by the Board via a red box. *Id.* “Z(F30A), and all mutants thereof includes the same N-terminal as Z(F30A)” and “Z(N23T) was constructed with the same N-terminal as Z.” *Id.*⁵ Figure 1B shows “[t]he three-dimensional structure of the Z domain”

⁵ The mutation N23T having a change in amino acid correlates with the amino acid N next to the “Helix 2” box of Figure 1A as annotated by Petitioner. *See* Pet. 13.

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and “the different substitutions are indicated.” *Id.* Specifically, Linhult discloses,

[t]he B domain has been mutated in order to achieve a purification domain resistant to cleavage by hydroxylamine. An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine.[] Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.[] Protein Z is well characterized and extensively used as both ligand and fusion partner in a variety of affinity chromatography systems.

Id. Using a 0.5 M NaOH cleaning agent and “a total exposure time of 7.5 h for Z(F30A) and mutants thereof,” Linhult determines that “N23 seems to be very important for the functional stability after alkaline treatment of Z(F30A)” and “Z(F30A,N23T) shows only a 28% decrease in capacity despite the destabilizing F30A-mutation.” *Id.* at 4–5; Figs. 2, 3. Linhult reports that “[h]ence, the Z(F30A,N23T) is almost as tolerant as Z and is thereby the most improved variant with Z(F30A) as scaffold.” *Id.* at 5; Figs. 2, 3.

Linhult further discloses that “Z, Z(F30A), and mutated variants were covalently coupled to HiTrap™ affinity columns,” that “[t]he Z domain includes 8 asparagines (N3, N6, N11, N21, N23, N28, N43, and N52; Fig. 1),” and that “since the amino acid is located outside the structured part of the domain, it will most likely be easily replaceable during a multimerization of the domain to achieve a protein A–like molecule.” *Id.* at 4. Linhult confirms that “the affinity between Z(F30A) and IgG was retained despite the mutation.” *Id.* In Linhult’s studies, “[h]uman polyclonal IgG in TST was prepared and injected onto the columns in excess” and “[a] standard affinity chromatography protocol was followed.” *Id.*

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2. *Abrahmsén (Ex. 1005)*

Abrahmsén “relates to a recombinant DNA fragment coding for an immunoglobulin G ([I]gG) binding domain related to staphylococcal protein A . . . and to a process for cleavage of a fused protein expressed by using such fragment or sequence.” Ex. 1005, 1:8–13. Abrahmsén discloses that “[b]y making a gene fusion to staphylococcal protein A any gene product can be purified as a fusion protein to protein A and can thus be purified in a single step using IgG affinity chromatography.” *Id.* at 1:22–26. Abrahmsén explains that Protein A has “5 Asn-Gly in the IgG binding region of protein A” which “makes the second passage through the column irrelevant as the protein A pieces released from the cleavage will not bind to the IgG.” *Id.* at 1:58–63. Abrahmsén provides a solution to this problem “by adapting an IgG binding domain so that no Met and optionally no Asn-Gly is present in the sequence.” *Id.* at 1:64–67.

Abrahmsén discloses that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “[t]he number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén discloses that the recombinant DNA fragment can “cod[e] for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.” *Id.* at 2:32–37. According to Abrahmsén, from a computer simulation of the Gly to Ala amino acid change, it was “concluded that this change would not interfere with folding to protein A or binding to IgG.” *Id.* at 5:13–16.

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3. *Hober (Ex. 1006)*

Hober “relates to . . . a mutant protein that exhibits improved stability compared to the parental molecule” and “also relates to an affinity separation matrix, wherein a mutant protein according to the invention is used as an affinity ligand.” Ex. 1006, 1. Hober discloses that removal of contaminants from the separation matrix involves “a procedure known as cleaning-in-place (CIP)” but “[f]or many affinity chromatography matrices containing proteinaceous affinity ligands,” the alkaline environment “is a very harsh condition and consequently results in decreased capacities owing to instability of the ligand.” *Id.* at 1–2. According to Hober, structural modifications, such as deamidation and cleavage of the peptide backbone, of asparagine and glutamine residues in alkaline conditions is the main reason for loss of activity in alkaline solutions and that “the shortest deamidation half time have been associated with the sequences -asparagine-glycine and -asparagine-serine.” *Id.* at 2. Further, from a study of a mutant of ABD that was created, it was concluded that “all four asparagine residues can be replaced without any significant effect on structure and function.” *Id.* at 2–3. Hober points out that the SPA contains domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species and reagents of this protein with their high affinity and selectivity have found a widespread use in the field of biotechnology. *Id.* at 3. Accordingly, “there is a need in this field to obtain protein ligands capable of binding immunoglobulins, especially via the Fc-fragments thereof, which are also tolerant to one or more cleaning procedures using alkaline agents.” *Id.* at 4.

In one embodiment of Hober, a multimer “comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A” in which

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“asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids” for advantageous structural stability reasons wherein “the glycine residue in position 29 of SEQ ID NO: 1 has also been mutated, preferably to, an alanine residue.” *Id.* at 12. Hober’s SEQ ID NO: 1 is reproduced below.

Ala	Asp	Asn	Lys	Phe	Asn	Lys	Glu	Gln	Gln	Asn	Ala	Phe	Tyr	Glu	Ile
1				5					10					15	
Leu	His	Leu	Pro	Asn	Leu	Asn	Glu	Glu	Gln	Arg	Asn	Gly	Phe	Ile	Gln
			20					25					30		
Ser	Leu	Lys	Asp	Asp	Pro	Ser	Gln	Ser	Ala	Asn	Leu	Leu	Ala	Glu	Ala
		35					40					45			
Lys	Lys	Leu	Asn	Asp	Ala	Gln	Ala	Pro	Lys						
	50					55									

Id. at SEQUENCE LISTING 1. SEQ ID NO: 1 shows a domain of *Staphylococcus aureus* having Glycine (Gly) as an amino acid at the position 29, as annotated via red highlighting.

Hober further discloses that its matrix for affinity separation “comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which protein at least one asparagine residue has been mutated to an amino acid other than glutamine.” *Id.* at 13. For its method of isolating an immunoglobulin, Hober discloses “in a first step, a solution comprising the target compounds, . . . is passed over a separation matrix under conditions allowing adsorption of the target compound to ligands present on said matrix” and “[i]n a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption, i.e. release of the target compound.” *Id.* at 16.

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E. *Obviousness in view of Linhult, Abrahmsén, and Hober*

1. *Petitioner's Contentions*

a) *Claims 1 and 14⁶*

Petitioner argues that “*Linhult* explains that SPA-based chromatography matrices have ‘widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.’” Pet. 19 (citing Ex. 1004, 1). Petitioner argues that *Linhult* teaches using a HiTrap™ chromatography affinity column made up of agarose beads that serve as a solid support for coupling SPA-based ligands. *Id.* (citing Ex. 1004, 4; Ex. 1002 ¶ 86). “*Linhult* discloses that its SPA-based ligands were ‘coupled to’ the solid support agarose beads [] contained in HiTrap™ affinity columns.” *Id.* at 20 (citing Ex. 1004, 4). Petitioner argues that “*Linhult* discloses that ‘multimerization’ of SPA monomers is performed to ‘achieve’ an “[SPA-]like’ affinity ligand.” *Id.* (citing Ex. 1004, 4; Ex. 1002 ¶¶ 91–93). Petitioner argues that “Figure 1(a), *Linhult* describes at least 55 amino acids of SPA’s naturally-occurring C domain (i.e., SEQ ID NO. 1).” *Id.* (citing Ex. 1004, 1, Fig. 1(a); see Ex. 1005, Fig. 2; Ex. 1006, Fig. 1; Ex. 1008, 639, Fig. 1). Petitioner argues “that all ‘five SPA domains show individual affinity for the Fc-fragment . . . as well as certain Fab-fragments of [antibodies] from most mammalian species.” *Id.* at 21 (citing Ex. 1004, 1). Petitioner argues that “*Abrahmsén* teaches a C(G29A)-based SPA ligand.” *Id.* (citing Ex. 1002 ¶¶ 99–111).

⁶ Petitioner treats claims 1 and 14 and their corresponding depend claims similarly; therefore, our analysis in this Decision groups the claims together.

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Petitioner argues that it was “known that individual SPA domains, including the C domain, could be used to construct SPA-based affinity ligands for purifying proteins.” *Id.* at 21 (citing Ex. 1002 ¶¶ 29, 101; Ex. 1004, 1; Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). Petitioner argues that “Linhult thus teaches a [person of ordinary skill in the art] that avoiding the Asn₂₈-Gly₂₉ dipeptide sequence through a G29A mutation, including on the C domain, would yield an SPA-based ligand having increased alkali-stability.” *Id.* at 22 (citing 1002 ¶¶ 100–04; Ex. 1011; Ex. 1012; Ex. 1013). Petitioner acknowledges that Linhult “does not expressly disclose a C(G29A)-based SPA ligand. Regardless, it would have been obvious to a [person of ordinary skill in the art] to modify *Linhult* based on the teachings of *Abrahmsén* to incorporate a C(G29A)-based SPA ligand in a chromatography matrix.” *Id.* at 23 (Ex. 1002 ¶¶ 99–111). Petitioner argues “*Abrahmsén* expressly discloses ‘a recombinant DNA coding for any of the E D A B C domains of [SPA], wherein the glycine codon(s) in the Asn_[28]-Gly_[29] coding constellation has been replaced by an alanine codon.’” *Id.* at 23 (citing Ex. 1005, 2:32–37).

Petitioner concludes that

applying the teachings of *Abrahmsén* with *Linhult* would have involved merely combining known elements in the field (e.g., an affinity chromatography matrix comprising a G29A-containing ligand coupled to a solid support, as in *Linhult*, and a C(G29A)-based amino acid sequence, as in *Abrahmsén*) according to known ligand-construction methods to yield a predictable results (e.g., the claimed affinity chromatography matrix).

Pet. 24.

Petitioner argues that Abrahmsén “unequivocally discloses including a G29A mutation on SPA’s C domain.” Pet. 49 (citing Ex. 1005, 2:32–37,

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5:13–16; Ex. 1002 ¶¶ 222–27). The Petition recognizes that in Abrahmsén an IgG chromatography column was used to purify the recombinantly produced SPA. *Id.* In other words, the column matrix had IgG bound to the matrix and could capture the recombinantly produced SPA binding domains.

Petitioner relies on Hober for teaching that the substitution in G29A of SEQ ID NO: 1 would have been obvious and to address the conventional features set forth in dependent claims. *See* Pet. 20–26, 48–49. Specifically, Petitioner argues that Hober teaches “that naturally-occurring SPA ‘contain[s] domains capable of binding to the Fc and Fab portions’ of antibodies.” Pet. 33 (citing Ex. 1006, 3); Ex. 1006, 3 (“An example of such a protein [used for affinity chromatography] is staphylococcal protein A, containing domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species.”). Petitioner argues that Hober also teaches that the shortest deamidation half lives are seen with sequences that contain an Asn-Gly dipeptide. Pet. 33 (citing Ex. 1005, 2); Ex. 1002 ¶ 76 (“*Hober* teaches that Asn-Gly dipeptide sequences are associated with ‘the shortest deamidation half times,’ which is a degradation process that occurs at high pH conditions.”), *see id.* ¶ 141 (deamidation “is a process that degrades SPA-based ligands at high pH conditions.”).

According to Petitioner, “*Hober* discloses, for example, that ‘SPA-based affinity medium probably is the most widely-used affinity medium for isolation of monoclonal antibodies and their fragments from different samples.’” Pet. 50 (citing Ex. 1006, 3).

Hober states, for example, that SPA-based monomers “can be combined into multimeric proteins, such as dimers, trimers, tetramers, pentamers etc.” (Ex. 1006, 11; *see also* Ex. 1005, 9:14–10:41 (*Abrahmsén* disclosing Example V on “Construction

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of Dimeric Z Fragment”).) A [person of ordinary skill in the art] would have a good reason to combine the teaching from *Abrahmsén* with the teachings of *Hober* since multimeric SPA-based ligands allowed for additional surface area to bind target compounds. (Ex. 1002 ¶ 239.)

Pet. 52–53. Petitioner’s declarant, Dr. Cramer explains that using the ligand as a multimeric ligand has “the benefits of additional surface area on the SPA-based ligand to bind target compounds.” Ex. 1002 ¶ 239 (citing Ex. 1032, 560). *Abrahmsén* discloses that SPA domain C as shown in Figure 2 (*see* Ex. 1005 at col. 3:25-35, Fig. 2) has at least 55 contiguous amino acids of SEQ ID NO. 1. *See* Ex. 1002 ¶¶ 242–244. “*Abrahmsén* further confirms there are no structural integrity concerns in constructing a C(G29A)-based SPA ligand by disclosing that incorporation of a G29A mutation ‘would not interfere with folding [of SPA] or binding to [antibodies].’” Ex. 1002 ¶ 246.

b) Claims 4 and 17

With respect to claims 4 and 17, Petitioner argues that the property of being “capable” of binding to the Fab fragment of an antibody is an inherent property of SEQ ID NO:1 that has the glycine exchanged for the alanine at position 29. *See* Pet. 28. Specifically, Petitioner argues that “*Linhult* discloses that it was well known by 2004 that the five domains of SPA, including the C domain, ‘show individual *affinity for*...certain *Fab-fragments* of [antibodies] from most mammalian species.’” Pet. 29 (citing Ex. 1004, 1; Ex. 1002 ¶ 125). “Even if the capability to bind the Fab part of an antibody is not an inherent property of the claimed C(G29A)-based SPA ligand, *Linhult* discloses this limitation.” *Id.* Petitioner notes further that “dependent claims 4 and 17 permit the incorporation of additional amino

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acid sequences known to possess the claimed property.” *Id.* (citing Ex. 1002 ¶ 126).

c) Claims 2, 3, 12, 15, 16, and 25

With respect to claims 2, 3, 12, 15, 16, and 25, Petitioner directs our attention to where in the record the various limitations of the dependent claims may be found. *See* Pet. 25–28, 30. Specifically, Petitioner asserts that Linhult describes multimerization of the ligand as recited in claims 2, 12, 15, and 25. Pet. 26 (citing Ex. 1004, 410; Ex. 1002 ¶¶ 112–114), *see id.* at 30 (citing Ex. 1002 ¶¶ 128–129). Because the wild-type C-domain-based multimeric ligand inherently possesses the claimed binding-capacity property as recited in claims 3 and 16, there is a reasonable expectation that a chromatography possessing the C(G29A)-mutation would achieve similar binding capacity. Pet. 27–28 (citing Ex. 1002 ¶¶ 115–119).

2. Patent Owner’s Contentions

Patent Owner argues that the Petition fails to demonstrate that it would have been obvious to make the chromatography matrix as claimed (PO Resp. 17–38); that the Petition has not established that there is a reasonable expectation of success in arriving at the claimed matrix (*id.* at 47–52); that the art teaches away from making the G29A modification (*id.* at 38–41); that the artisan would not have been motivated make additional mutations (*id.* at 43–44); and that objective indicia supports a conclusion of non-obviousness (*id.* at 53–53).

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a) Matrix

According to Patent Owner, “Petitioners fail to explain why the POSA would have been motivated to select Domain C’s amino acid sequence as the foundation for an engineered SPA ligand with favorable properties.”

PO Resp. 18. Specifically arguing that the obviousness analysis requires the prior art be viewed as a whole. PO Resp. 20 (citing *In re Wesslau*, 353 F.2d 238 (CCPA 1965); *In re Enhanced Sec. Rsch., LLC*, 739 F.3d 1347, 1355 (Fed. Cir. 2014); *Impax Lab ’ys Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018)).

Patent Owner argues that because nobody was working on Domain C at the time the invention was filed, therefore, the selection of Domain C for further development could not possibly be obvious. *See* PO Resp. 23 (“Reliance on *KSR* also is foreclosed by the evidence that no one in the art was seeking to modify Domain C.”), *see also id.* at 24 (“But no prior art cited by Petitioners singles Domain C out for further development. Ex. 2025 ¶¶ 89-96”), *id.* at 26 (“Dr. Cramer [Petitioner’s expert] himself highlights, it would have been natural for the POSA to further develop the domain—Domain B—that was best understood and for which there was a crystal structure available. Ex. 1002 ¶ 33; Ex. 2015 at 137:20–138:19; Ex. 2017”), *id.* at 28 (“The notion that this body of work would lead the POSA to discard the improved ligands the references themselves focus on, and instead start experimenting with mutations to Domain C—strains credulity. Ex. 2025 ¶¶ 92–95”).

According to Patent Owner, neither Linhult nor Abrahmsén supply the motivation to start with Domain C. “Linhult focuses exclusively on, and concerns improvements to, the alkaline stability of Domain Z by mutating

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asparagine residues. *See* Ex. 2025 ¶¶ 64-67, 92.” PO Resp. 29. “Rather than use Domain C, the POSA reviewing Linhult would be motivated to keep working with Domain Z, adopting the N23T mutation. Ex. 2025 ¶ 67 & n.3.” PO Resp. 30. “Neither Abrahmsén itself nor the Petition provide any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domains. *WBIP[LLC v. Kohler Co.]*, 829 F.3d 1317, 1337 (Fed. Cir. 2016)].” PO Resp. 31.

b) Reasonable Expectation of Success

Patent Owner argues that “the field of protein engineering is notoriously unpredictable.” PO Resp. 21 (citing Ex. 2025 ¶¶ 50-52). Arguing that “despite their supposed structural similarity, there are a number of differences between the naturally-occurring domains of protein A, including five different amino acids in the sequences of Domain B (with which the industry was quite familiar) and Domain C (which remained virtually ignored as of the priority date).” *Id.* at 22 (citing Ex. 2025 ¶ 48).

Protein engineering is a highly complex and unpredictable field and was all the more so as of the priority date more than fifteen years ago. *See, e.g.,* Ex. 2025 ¶¶ 50-52. . . . As amply demonstrated by the effect of the G29A mutation on Domain Z’s Fab-binding ability, even a single amino acid substitution can drastically alter the properties of a protein. Ex. 2025 ¶ 52; Ex. 2015 at 51:15-52:1 ([Dr. Cramer, Petitioner’s expert] agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability), 18:10-12, 73:16-20.

PO Resp. 34–35.

Patent Owner argues that

The Federal Circuit has rejected arguments premised on the notion that a homologous structure renders an invention obvious, particularly given the difficulty and uncertainty in the art as of

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the priority date. *See, e.g., Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208 (Fed. Cir. 1991) (holding the use of a monkey gene to probe for a roughly 90 percent “homologous” human gene would not have been obvious, particularly given expert testimony that isolating a particular gene would have been “difficult” and the lack of certainty in the endeavor).

PO Resp. 36. Specifically, Patent Owner argues that the Fab-binding capability of a ligand could not have been predicted and therefore there is no reasonable expectation of success. *See* PO Resp. 38.

c) Teaching Away

Patent Owner argues that the prior art would have told the person of ordinary skill in the art to avoid a G29A a modification to Domain C.

PO Resp. 38. In other words, its Patent Owner’s contention is that the prior art teaches away from making this modification. “The very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding ‘negligible’ when implemented in Domain B.” PO Resp. 40. Patent Owner argues that a person seeking to improve Fab binding one avoid a G29A substitution of Domain C. PO Resp. 40–41.

d) Additional Modifications

Patent Owner argues that “the prior art would have taught the POSA to make asparagine substitutions, not glycine substitutions, to address alkaline stability concerns.” PO Resp. 43. In other words, Patent Owner’s argues the prior art would have suggested making additional substitutions most notably in the asparagine residues of Domain C. *Id.* at 44.

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e) Inherency

Patent Owner argues that Petitioner cannot rely on inherency to circumvent that the combination lacks a reasonable expectation of success. PO Resp. 52 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. DE C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017)). “As Dr. Bracewell [Patent Owner’s expert] explains, the POSA did not know and could not have known the properties of a modified Domain C ligand, since the effects of even single-amino acid substitutions are undisputedly unpredictable and the cited art includes absolutely no protein engineering work with Domain C. Ex. 2025 ¶¶ 120-122.” PO Resp. 48. According to Patent Owner, because nobody was working on domain C the person of ordinary skill in the art “is simply left to guess at how such a ligand would perform.” PO Resp. 49 (citing Ex. 2025 ¶¶ 121–124). Specifically, the person of ordinary skill in the art would not know whether a Domain C-G29A mutation would be capable of binding Fab part of an antibody. *Id.*

f) Unexpected Results

Patent Owner argues that it was wholly unexpected that the modified C(G29A)-based SPA ligand to bind the Fab part. PO Resp. 55. “[T]he SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” PO Resp. 55.

3. Petitioner’s Reply

In response, Petitioner argues that

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Abrahmsén and *Hober* each expressly pointed to a C(G29A) mutation (Ex. 1005, 2:32-37; Ex. 1006, 12), which was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence (*see, e.g.*, Ex. 1004, 408). As the Board recognized, “*Abrahmsén* provides motivation for making [the G29A] mutation in **any of the IgG binding domains**.” (Decision, 24; *see also* Ex. 1057, 97:3-16 (Dr. Bracewell admitting that *Abrahmsén* discloses a G29A mutation to any of the five domains, including Domain C).)

Reply 2.

A POSA would have reasonably expected success in combining these teachings to achieve the claimed affinity chromatography matrix given the well-known fact that each individual domain, including Domain C, has affinity for antibodies (Ex. 1004, 407), as well as *Abrahmsén*’s confirmation that G29A “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1057, 99:13-101:21 Ex. 1005, 5:13-16; Ex. 1002 ¶110).

Id. at 3.

Petitioner argues that Patent Owner “has not disputed that *Abrahmsén* disclosed that G29A ‘would not interfere with folding to protein A or binding to IgG.’ (Ex. 1005, 2:32-37, 5:4-16; Ex. 1057, 109:20-110:17.) Nor does it take issue with its own statements in *Hober* that G29A is advantageous for ‘structural stability reasons.’ (Ex. 1006, 12.)” *Id.* at 9. “*Abrahmsén* and *Hober*, which make clear that G29A does not affect the ability of an SPA ligand to bind to an antibody. (Ex. 1005, 2:32-37, 5:4-16; Ex. 1006, 12.)” *Id.* at 10.

Petitioner argues that “a POSA would have started with any one of the naturally occurring domains. (Decision, 26-28.) To then increase alkali stability, a POSA would have made the simplest, well-known substitution: G29A. (Section II.A.1-2; Ex. 1061 ¶¶8-15.)” Reply 11.

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Petitioner argues “that Fab-binding was an inherent feature of a C(G29A)-based ligand—which [Patent Owner] does not appear to dispute. (Decision, 30; [PO] Resp., 52-53.) In fact, [Patent Owner] acknowledges that ‘C(G29A)-based SPA ligands retained substantial Fab-binding ability.’ (Resp., 55.)” *Id.* at 14.

Petitioner argues that “Fab-binding is not being used [in the Petition] as part of a finding of a motivation to combine; rather, it is an inherent property [of the composition] being claimed. And necessarily present properties do not add patentable weight when they are claimed as limitations. *In re Kubin*, 561 F.3d 1351, 1357 (Fed. Cir. 2009).” Reply 15–16. Petitioner further argues that Patent Owner’s reliance on *Honeywell* is misplaced because “*Honeywell* had to do with an inherent property being used as a teaching in an obviousness analysis; it did not involve a limitation in the challenged claim reciting an inherent property.” Reply 15 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. De C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017); *see also Pernix Ireland Pain v. Alvogen Malta Operations*, 323 F. Supp. 3d 566, 607(D. Del. 2018)).

4. Patent Owner’s Sur-reply

Patent Owner argues that “Petitioners, and the Institution Decision, overlook an important point of consensus between the parties’ experts: the field of protein engineering is notoriously *unpredictable*.” Sur-reply 2. Patent Owner maintains that Petitioner has not identified a motivation to start from Domain C. *Id.* at 3. Patent Owner argues that “Petitioners would have the Board look past the multitude of references teaching a preference for Domains B and Z—including Petitioners’ foundational references—and

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seize upon fleeting mentions of Domain C.” *Id.* at 7 (citing *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965)). Patent Owner argues that “[m]ere sequence homology does not make the field predictable, as both experts observe, Ex. 2015 at 51:15-52:1, 56:4-12, 75:12-22, 73:16-20; Ex. 2025 ¶¶ 50-52; Ex. 2049 at 72:1-73:12, and as the vastly different Fab-binding properties of the near-identical Domains B and Z well illustrate, Ex. 2029 at 8.” *Id.* at 8–9.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

5. Analysis

a) Claims 1 and 14

Claims 1 and 14 of the ’765 patent are directed to a composition. Specifically, a chromatography matrix (i.e. a solid support) that has a ligand attached, and that ligand is made up of at least two polypeptides comprising 55 contiguous amino acids of SEQ ID NO: 1⁷ having a G29A mutation. *See* Ex. 1001, 15:39–48.

Linhult teaches that SPA is a cell surface protein expressed by *Staphylococcus aureus* and consists of five highly homologous domains (EDABC). Ex. 1004, 1. Each of “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as

⁷ Wild type amino acid sequence of domain C from *Staphylococcus* protein A (SPA). *See* Ex. 1001, 4:27, 6:35–36, 6:51–52.

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well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” *Id.* “Due to the high affinity and selectivity of SPA, it has a widespread use as an affinity ligand for capture and purification of antibodies.” Ex. 1004, Abst., *see also id.* at 1 (“SPA has a widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.”).

Linhult explains that, in column chromatography, sodium hydroxide (NaOH) is probably the most extensively used cleaning agent for removing contaminants such as nucleic acids, lipids, proteins, and microbes, and a cleaning-in-place (CIP) step is often integrated in the protein purification protocols using chromatography columns. Ex. 1004, 1. “Unfortunately, protein-based affinity media show high fragility in this extremely harsh environment, making them less attractive in industrial-scale protein purification. SPA, however, is considered relatively stable in alkaline conditions.” *Id.* at 2. Linhult teaches that the combination of “[a]sparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.” *Id.* Linhult teaches that “[a]n exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is [also] a cleavage site for hydroxylamine.” *Id.*

Petitioner’s declarant, Dr. Cramer explains that the “Z” domain referenced in Linhult refers to a synthetic version of the wild-type (i.e., natural) B domain of SPA, in which the naturally occurring glycine in the Asn₂₈-Gly₂₉ dipeptide sequence is replaced by an alanine residue to create an Asn₂₈-Ala₂₉ dipeptide sequence. Ex. 1002 ¶ 30 (citing Ex. 1004, 408, Fig. 1(a); Ex. 1007, 109, Fig. 1); ¶ 31 (citing Ex. 1005).

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We credit Dr. Cramer for establishing that the C domain sequence disclosed in Linhult contains 55 amino acids of domain C as recited in SEQ ID NO: 1. Ex. 1002 ¶ 97 (showing a sequence alignment), *see also id.* ¶ 243 (showing sequence alignment of domain C of Abrahmsén with SEQ ID NO: 1); *see also* Ex. 2025 ¶ 41 (Patent Owner’s declarant Dr. Bracewell showing SPA sequence alignment for Domains A, B, C, D, E, and Z).

Linhult teaches making affinity chromatography columns with protein Z, Z(F30A), and additional mutated variants. These modified proteins were covalently attached to HiTrap™ columns in Linhult using NHS-chemistry. Ex. 1004, 4. Human polyclonal IgG was prepared and injected onto the columns in excess and “[a] standard affinity chromatography protocol was followed.” *Id.* at 4. Linhult exemplifies using the Z domain and Z domain mutants attached to the column for the isolation of IgG from a sample. *Id.* Linhult, therefore, teaches column chromatography matrix that has a SPA domain containing a G29A mutation attached to a chromatography matrix.

According to Abrahmsén, the IgG binding domains of E D A B C domains of SPA were known. *See* Ex. 1005, 3:25–35, 4:34–37, Fig. 2. Abrahmsén teaches that the Asn-Gly dipeptide is located in the middle of an alpha helix involved in the binding to IgG. *Id.* 4:56–60; Ex. 2025 ¶ 41 (showing alpha helix regions in SPA domains). Relying on “computer analysis [Abrahmsén] surprisingly showed that the Gly in the Asn-Gly dipeptide sequence could be changed to an Ala. This change was not obvious as glycines are among the most conserved amino acids between homologous protein sequences due to their special features.” Ex. 1005, 5:7–9. Abrahmsén teaches that “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23.

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Abrahmsén teaches “a recombinant DNA fragment coding for *any of the E D A B C domains* of Staphylococcal protein A, wherein the *glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.*” *Id.* at 2:33–37 (emphasis added). Abrahmsén, therefore, provides motivation for making this mutation in *any one* of the IgG binding domains of E D A B C domains of SPA.

Abrahmsén, like Linhult, only exemplifies the cloning and expression of the Z-domain, which is a B-domain with a G29A mutation. Ex. 1005, 7:65–10:56. A references disclosure, however, is not limited only to its preferred embodiments, but is available for all that it discloses and suggests to one of ordinary skill in the art. *In re Lamberti*, 545 F.2d 747, 750 (CCPA 1976); *see also In re Susi*, 440 F.2d 442, 446 n.3 (CCPA 1971) (disclosed examples and preferred embodiments do not constitute a teaching away from a broader disclosure or non-preferred embodiments). Here, Abrahmsén expressly suggests making the same mutation in *any one* of the SPA domains E D A B C. Ex. 1005, 2:33–37

Thus, the disclosures of both Linhult and Abrahmsén suggest mutating the glycine at position 29 for an alanine in an any one of the SPA IgG binding domains of E D A B or C in order to avoid protein degradation in alkaline conditions and degradation by hydroxylamine. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”); Ex. 1005, 4:56–57 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine.”); Ex. 1006, 2 (“and the shortest deamidation half times

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have been associated with the sequences –asparagine–glycine and –asparagine–serine”); Reply 3–5; Ex. 1061 ¶¶ 3–6.

Based on these disclosures, the combined teachings of Linhult and Abrahmsén suggest making the G29A mutation in *any one* of the SPA IgG binding domains E, D, A, B, or C. We, therefore, agree with Petitioner that attaching any other mutated SPA IgG binding domains E, D, A, or C using “known ligand-construction methods to yield a predictable result[] (e.g., the claimed affinity chromatography matrix)” would have been obvious. Pet. 24 (citing Ex. 1002 ¶ 109). As the Federal Circuit has explained, “[w]here a skilled artisan merely pursues ‘known options’ from ‘a finite number of identified, predictable solutions,’ the resulting invention is obvious under Section 103.” *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421).

Accordingly, we agree with Petitioner that the combination of Linhult and Abrahmsén expressly suggests mutating the glycine codon for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111).

We address Patent Owner’s contentions below.

(1) Matrix

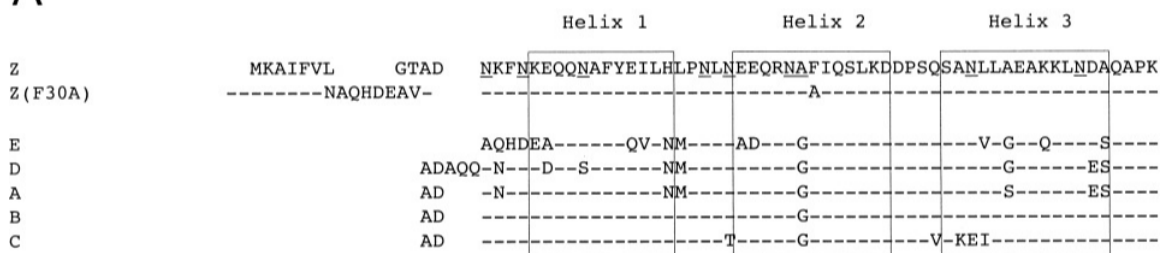
We do not find Patent Owner’s argument that the Petition fails to identify a reason to select domain C persuasive. PO Resp. 17–30. Specifically, we are not persuaded by Patent Owner’s contention that just because nobody was working on Domain C at the time the invention was filed, therefore, selection of Domain C cannot be obvious. *See* PO Resp. 31

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(“A general recognition that there exist five naturally occurring protein A domains is not a motivation to use each of them as a starting point for the claimed mutations”).

Petitioner’s articulated obviousness ground is premised on the knowledge that *any one* of the five SPA IgG binding domains are known to bind IgG and can function as a ligand for the purification of antibodies. Linhult and Abrahmsén both expressly suggest that the glycine codon at position 29 can be mutated for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Ex. 1004, 2; Ex. 1005, 2:32–37. The SPA IgG binding domains comprise a short list of 5 members: E, D, A, B, or C. Of these 5 members, the glycine at position 29 in Domain B has already been mutated to an alanine to create a Domain Z which has been shown to retain IgG binding activity. Ex. 1004, 6 (Fig. 3). Figure 1A of Linhult is reproduced below.

A



Linhult’s Figure 1A, reproduced above, shows the amino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C). The three boxes show the α -helices. Ex. 1004, 2; Ex. 1005, Fig. 2.

As discussed in our Institution Decision (Dec. 26–27), “it is fair to say that there were ‘a finite number of identified, predictable solutions’ to the problem of finding” a SPA IgG binding domain that is resistant to protein degradation by mutating the glycine at position 29 for an alanine and this is

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a “product not of innovation but of ordinary skill and common sense.” *See Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421). It is well established that

[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties.

In re Deuel, 51 F.3d 1552, 1558 (Fed. Cir. 1995). Here, Linhult and Abrahmsén show that the IgG binding domains of SPA – E, D, A, B, or C share many structural similarities. *See* Ex. 1004, 2 (Fig. 1(a) (reproduced above)); Ex. 1005, 3:25–35.

There is also an express teaching in both Linhult and Abrahmsén to mutate the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which supports the obviousness of incorporating the mutation into any IgG binding domain that has the Asn-Gly dipeptide. *See, e.g., SIBIA Neurosciences, Inc. v. Cadus Pharm. Corp.*, 225 F.3d 1349, 1358–59 (Fed. Cir. 2000) (stating that an express teaching in the prior art suggesting a particular modification establishes obviousness).

Accordingly, we find that Petitioner has shown by a preponderance of the evidence that the combined teachings of Linhult and Abrahmsén suggests the use of *any one* of the SPA IgG binding domains E, D, A, B, or C as the starting ligand for purifying IgG antibodies, and that making the G29A mutation in *any one* of the domains would have been obvious because it would provide ligands that are less susceptible to alkaline conditions and

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are resistant to hydroxylamine cleavage. Pet. 21–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

(2) Reasonable Expectation of Success

We are not persuaded by Patent Owner’s contention that there is no reasonable expectation of success in using a G29A mutation in Domain C. PO Resp. 39–40; 47–53.

Linhult explains that removing the asparagine–glycine amino acid combination not only results in the removal of the hydroxylamine cleavage site but also creates a product that is more alkaline resistant. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”).

Abrahmsén teaches that this Asn-Gly amino acid combination is present in all five IgG binding domains and that mutating the dipeptide would not interfere with IgG binding. Ex. 1005, 4:56–58 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine. As this sequence is kept intact in all five IgG binding domains of protein A. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”).

Abrahmsén’s conclusion that the mutation would not interfere with binding to IgG is supported by Linhult (*see* Ex. 1004, 6 (Fig. 3)) and Jansson.⁸

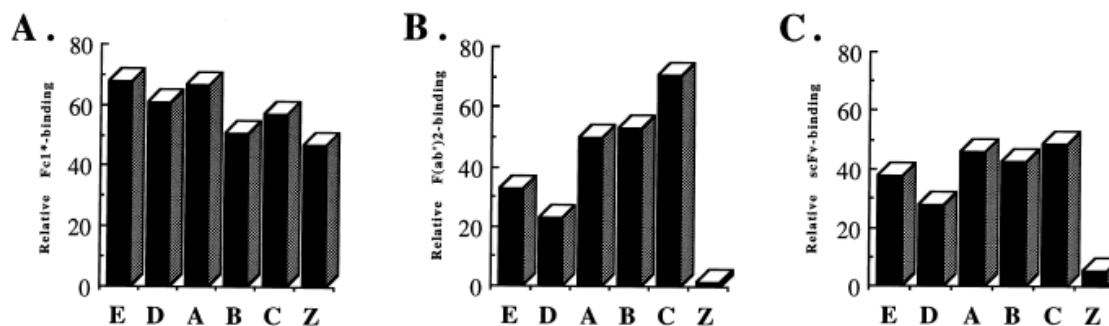
⁸ Patent Owner cites Jansson for the position that Domain Z has negligible binding to Fab. *See* PO Resp. 39–40. Claims 1 and 14 are not limited to Fab binding, indeed the claims do not even require IgG binding.

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Jansson's Fig. 3, reproduced below, shows a side-by-side comparison of Fc1*, Fab, and scFv binding.



Jansson Figure 3 (Panel A), reproduced above, shows that the single G29A mutation between Domain B and Domain Z results in a protein that is able to bind IgG.⁹ Comparing panel A–B column with panel A–Z column, the relative binding capacity in both columns remains close to 50% for both, indicating that the G29A mutation does not interfere with IgG binding. Ex. 2009, 6. This is a result already predicted by Abrahmsén's computer modeling and substantiated by Linholt. *See* Ex. 1005, 4:56–58; Ex. 1004, 6 (Fig. 3).

“Obviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success.” *In re Droge*, 695 F.3d 1334, 1338 (Fed. Cir. 2012) (quoting *In re Kubin*, 561 F.3d 1351, 1360 (Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894, 903–04

⁹ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the “IgG control” in Jansson. Patent Owner's counsel explains that “Part A is Fc binding. So that is, I believe the way they did this experiment was with Fc fragments, but it's generally acknowledged, you know, these antibodies all have an Fc domain if they're a whole antibody and that reflects the fact that all of these domains A, B, C, D and E and domain Z, which is B with the G29A mutation, retain this Fc binding.” Tr. 70:6–11.

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(Fed.Cir.1988)); *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016) (explaining that the expectation of success issue involves a showing of “a reasonable expectation of achieving *what is claimed*”) (emphasis added). It is not inventive to confirm something that was already suggested in the art. “Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention.” *Pharma Stem Therapeutic, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1363–1364 (2007).

Here, the record supports that each individual SPA domain, including the C domain, has affinity for IgG antibodies. Ex. 1004, 1 (“The five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” (bracketing in original)). Abrahmsén suggests making the mutation of Asn-Gly coding constellation in *any one* of the SPA domains by replacing glycine with an alanine codon that would remove the dipeptide sequence known to be sensitive to hydroxylamine degradation. *See* Ex. 1005, 4:56–5:16, *see also id.* Fig. 2 (showing the Asn-Gly coding constellation in all SPA domains); Ex. 1006, 2 (“the shortest deamidation half times have been associated with the sequences – asparagine–glycine and – asparagine–serine”). Abrahmsén’s confirms that a G29A mutation on SPA would not interfere with folding of SPA protein and the binding to antibodies. Ex. 1005, 5:13–16 (“by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”). Abrahmsén’s computer modeling suggests that IgG binding is not impacted by the mutation and this is confirmed by Linholt’s experiments showing that the

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G29A mutant of Domain B (a.k.a. Domain Z) binds IgG. Ex. 1004, 6 (Fig. 3).

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

We are not persuaded by Patent Owner’s contention that the information gained by computer modeling of the SPA native domain B – IgG crystal structure could not be extrapolated to other SPA domains that are structurally very similar.

As Petitioner’s expert, Dr. Cramer explains

It was well known that the researchers who developed the Z domain based on the wild-type B domain (rather than any of the other four SPA domains) did so for two reasons. (*See, e.g.*, Ex. 1007 at 109.) First, a crystal structure of the wild-type B domain binding to an antibody happened to be available in 1981 for analysis. (*See, e.g., id.*; Ex. 1005 at col. 4:56-68; Ex. 1017.) And, second, *their work would be informative of mutations that could be done on all five of the highly homologous SPA domains more generally.* (*See, e.g.*, Ex. 1005 at col. 2:32-37; Ex. 1007 at 109; Ex. 1008 at 639, Fig. 1.)

Ex. 1002 ¶ 33 (emphasis added). Dr. Cramer further explains that “[t]hey did the computer modeling based on that complex because that’s the crystal structure that they had. It wasn’t done because the B domain is special. . . . And then there’s several other places where they state clearly that they could also do the other domains with expected similar results.” Ex. 2015, 138:8–22.

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Accordingly, we agree with Petitioner that taken together, the teachings of Linhult, Abrahmsén, and Hober provide a reasonable expectation of success at arriving at a chromatography composition that contains the SPA domain C ligand with the G29A mutation. Pet. 25 (citing Ex. 1005, 5:13–16; Ex. 1002 ¶ 110).

(3) No Teaching Away

We are also not persuaded by Patent Owner’s contention that the art teaches away from the G29A substitution because it interferes with Fab binding. *See* PO Resp. 38–40; Sur-reply 9; Ex. 2009 at 2; Ex. 2010 at 25; Ex. 2012 at 25–26; Ex. 2029 at 7.

Neither claim 1 nor claim 14 recite a need to bind the Fab region of an antibody – all that is required by these claims is a chromatography matrix with a domain C ligand attached, and the domain C sequence having a G29A mutation.¹⁰ The law does not require that the teachings of the reference be combined for the reason or advantage contemplated by the inventor, as long as some suggestion to combine the elements is provided by the prior art as a whole. *In re Beattie*, 974 F.2d 1309, 1312 (Fed. Cir. 1992); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976); *see In re Kemps*, 97 F.3d 1427, 1430 (Fed. Cir. 1996) (“[T]he motivation in the prior art to combine the references does not have to be identical to that of the applicant to establish obviousness.”).

Here, Linhult teaches that “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most

¹⁰ We note that neither claim 1 nor claim 14 even requires IgG binding.

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mammalian species.” Ex. 1004, 1 (bracketing in original) (citation omitted). Linhult, therefore, teaches that *any one* of the SPA IgG binding domains E, D, A, B, or C can bind the Fc region of an antibody and can thereby be used as a ligand for purifying IgG antibodies. In addition, the combination of Linhult and Abrahmsén suggests making the G29A mutation in each of the domains because it would provide ligands that are less susceptible to protein degradation. Ex. 1004, 2; *see also* Ex. 1005, 2:33-37 (“[A] recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.”).

Patent Owner contends that the G29A mutation would lead to a reduction in the Fab binding of domain C, and therefore, would lead away from making the mutation. PO Resp. 40–41 (citing Ex. 2010 at 2; Ex. 2011 at 25; Ex. 2012 at 25–26; Ex. 2013 at 2–3; Ex. 2025 ¶¶ 105–109; Ex. 2029 at 6–7). Patent Owner’s cited references are directed to Fab binding. But claims 1 and 14 are not limited to Fab binding. Showing that the G29A mutation interferes with Fab binding says nothing about the ability of a mutated SPA domains E, D, A, B, or C to bind the Fc portion of IgG. *See, e.g.*, Ex. 2013, 3 (“The site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ^[11] binding.”).

¹¹ Fcγ is the constant region of IgG involved in effector function. Specifically, “[t]he Fcγ binding site has been localized to the elbow region at the CH2 and CH3 interface of most IgG subclasses, and this binding property has been extensively used for the labeling and purification of antibodies.” Ex. 2013, 1. In other words, Fcγ and Fc terminology are used interchangeably in the art.

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Accordingly, we are not persuaded by Patent Owner’s contention that the art teaches away from the combination as articulated by Petitioner.

(4) Additional Modifications

We are also not persuaded by Patent Owner’s contention the ordinary artisan would not stop with a single G29A mutation in a SPA domain. *See* PO Resp. 43–45. Here, Abrahmsén expressly suggests making only a single mutation. Specifically, Abrahmsén contemplates “a recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon” without additional mutations. Ex. 1005, 2:33–37.

(5) Summary

Having considered the evidence and argument cited by the Petition, which we adopt as our own, we are persuaded that Petitioner has shown by a preponderance of evidence of record that the combination of Linhult and Abrahmsén teach each of the limitations of claims 1 and 14. Petitioner not only has articulated a sufficient motivation for making the combination but has also established that there is a reasonable expectation of success for the binding of an IgG antibody to a SPA domain that contains an G29A mutation.

b) Claims 4 and 17

Petitioner argues that “[t]he ‘capab[ility] of binding to the Fab part of an antibody,’ as recited in claims 4 and 17, is an inherent property of the claimed C(G29A)-based SPA ligand.” Pet. 28 (citing Ex. 1002 ¶¶ 120–127).

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Petitioner contends that a person of ordinary skill in the art did not need to recognize the Fab binding property of Domain C to be motivated to select that domain for modification. “A POSA would not have been motivated only by Fab-binding ability,[] as even Dr. Bracewell agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’” Reply 8 (citing Ex. 1057, 75:17–76:4, 113:23–114:11, 157:24–158:9; Ex. 1061 ¶ 29).

Patent Owner argues that “[n]either Linhult itself nor the Petition provide any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domain sequences (or in the figure, those plus Domain Z’s sequence). PO Resp. 29 (citing *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1337 (Fed. Cir. 2016)); *see also* Sur-reply 1 (“Petitioners fail to show that the person of ordinary skill in the art (the ‘POSA’) would have plucked Domain C from the sea of prior art teaching a preference for Domains B and Z, substituted one and only one amino acid—the glycine at position 29 with an alanine (a ‘G29A’ mutation)—and used that mutated protein as a chromatography ligand for the purification of antibodies or antibody fragments.”).

Patent Owner argues that

[t]he very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding ‘negligible’ when implemented in Domain B. Ex. 2009 at 2; *see also, e.g.*, Ex. 2010 at 2 (“Fab binding activity is located to a region determined by helices 2-3, including the position mutated to yield the Z domain.”); Ex. 2011 at 25 (“[I]t only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine to an alanine”); Ex. 2012 at 25-26 (“[D]omain

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Z containing a single G29A-substitution compared to domain B exhibits little or no [Fab] binding. This might be due to the substitution since the C_β of the alanine would perturb the interaction between the two molecules.”).

PO Resp. 40.

Claim 1 is directed to a composition. Claim 4 is dependent on claim 1 and further recites “wherein the ligand is capable of binding to the Fab part of an antibody.” Ex. 1001, 15:56–57. The “capable of binding” language of claim 4, however, does not add any structural limitations to claim 1 it merely recites the function of the composition when used for example in an assay.¹²

“[T]erms [that] merely set forth the intended use for, or a property inherent in, an otherwise old [or obvious] composition . . . do not differentiate the claimed composition from those known in the prior art.” *In re Pearson*, 494 F.2d 1399, 1403 (CCPA 1974). “Inherency may supply a missing claim limitation in an obviousness analysis. An inherent characteristic of a formulation [i.e. composition] can be part of the prior art in an obviousness analysis even if the inherent characteristic was unrecognized or unappreciated by a skilled artisan.” *Persion Pharms. LLC v. Alvogen Malta Operations Ltd.*, 945 F.3d 1184, 1190 (Fed. Cir. 2019) (citations omitted).

As explained above (II.E.5.a), Abrahmsén teaches replacing the glycine codon in the Asn-Gly constellation in *any one* the SPA domains with an alanine codon. Ex. 1005, 2:21–23. Abrahmsén further explains that:

The *Asn-Gly dipeptide* sequence is sensitive to hydroxylamine. As *this sequence is kept intact in all five IgG*

¹² The same issue applies to claims 14 and 17. We note that none of the claims in the ’765 patent are directed to a method of using the composition in an assay to isolate Fab fragments.

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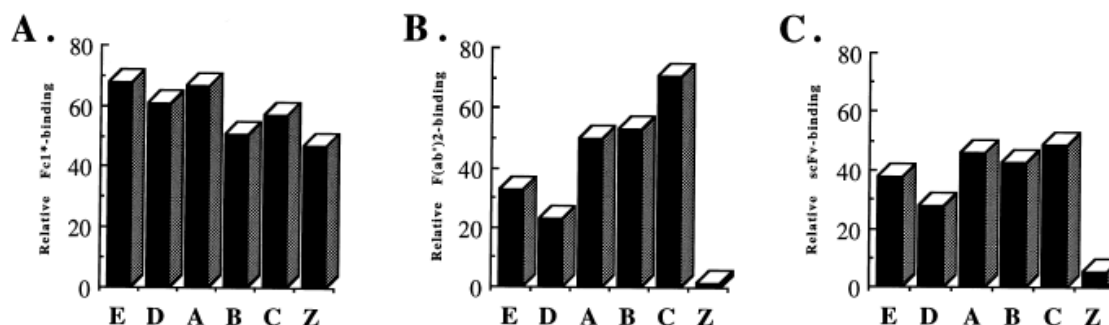
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binding domains of protein A and as this amino acid sequence is present in the middle of an alpha helix involved in the binding to IgG (FIG. 2) there is very little chance to be successful in any amino acid change. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.

Ex. 1005, 4:56–5:16 (emphasis added).

That the G29A mutation in domain B (resulting in domain Z) does not interfere with IgG binding was already established by Linhult. *See* Ex. 1004, 6 (Fig. 3 (showing IgG binding with domain Z)). The Domain Z binding property to IgG is further supported by Jansson. Jansson Fig. 3, reproduced below, shows the side-by-side comparison of Fc1*¹³, Fab, and scFv binding and confirms what was already suggested in Abrahmsén and Linhult – that a composition containing the G29A mutation in an SPA domain can bind IgG.



Panel A in Jansson Figure 3, reproduced above, shows that the single G29A mutation between Domain B and Domain Z does not result in the loss of IgG binding. Ex. 2029, 6 (Fig. 3). Because the other SPA domains are structurally so similar to Domain B there is a reasonable expectation that these domains would similarly retain the ability to bind IgG with the same G29A mutation. *See above* II.E.5.a.

¹³ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the IgG control in Jansson.

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We, therefore, agree with Petitioner that the limitation of the Fab binding ability as recited in claim 4 is an inherent feature of the structure disclosed in the independent claim 1. *See* Pet. 28 (citing Ex. 1002 ¶¶ 120–123). For the reasons disclosed above (II.E.5.a), we find that Petitioner has shown by a preponderance of evidence that there is a reasonable expectation that making a G29A mutation in *any one* of the SPA domains would result in a structure that retains the ability to bind IgG.

(1) Unexpected Results

Patent Owner argues that objective indicia of non-obviousness of the Domain C–G29A based ligands requires reaching a conclusion of non-obviousness. PO Resp. 53–55. Specifically, Patent Owner contends that “the SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” *Id.* at 55 (citing Ex. 2025 ¶ 123; Ex. 2030 at 18–19).

We are not persuaded by Patent Owner’s unexpected results argument. The prior art does not need to recognize that Domain C retains the ability to bind Fab fragments after a G29A mutation. Petitioner’s articulated rationale is that there is a reason to make the G29A mutations in *any one* of the SPA domains in order to get a product that is alkaline stable. Pet. 21–22 (citing Ex. 1002 ¶¶ 21, 99–11; Ex. 1004, 407–408, Fig. 1(a); Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). We find that Petitioner has shown by a preponderance of evidence that there is a reasonable expectation that making a G29A mutation in *any one* of the SPA domains results in a product that binds at least IgG. *See above* II.E.5.a. There is no requirement that the

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inherent characteristic of the Fab binding needed to be recognized in order to arrive at the conclusion that the recited structure in claim 1 would have been obvious. *See Persion Pharms.*, 945 F.3d at 1190 (“Our predecessor court similarly concluded that it ‘is not the law’ that ‘a structure suggested by the prior art, and, hence, potentially in the possession of the public, is patentable ... because it also possesses an [i]nherent, but hitherto unknown, function which [the patentees] claim to have discovered.’ *In re Wiseman*, 596 F.2d 1019, 1023 (C.C.P.A. 1979).”).

c) Claims 3 and 16

Claim 3 depends from claim 1, and claim 16 depends from claim 14. Claims 3 and 16 recite the additional limitation “wherein the chromatography matrix has retained at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.” Ex. 1001, 15:52–56. We find Petitioner has shown by a preponderance of the evidence that the combination of Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims for the reasons stated in the Petition which we adopt as our own. *See* Pet. 25–28, 30; Reply 20–21.

We are not persuaded by Patent Owner’s argument that “none of Petitioners’ cited references actually describe a C(G29A)-based SPA ligand, let alone provide alkaline stability data or test results concerning the same, the POSA is simply left to guess at how such a ligand would perform.” PO Resp. 49 (citing Ex. 2025 ¶¶ 121–124).

Petitioner has shown by a preponderance of the evidence of record that there is a reason for making the G29A mutation in *any one* of the four remaining SPA domains in order to produce a SPA product that is more

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alkaline stable and would reasonably bind IgG. We, therefore, agree with Petitioner, that the ability to retain the requisite binding capacity after a period of exposure to alkaline treatment is a property of the composition. Pet. 25–28; *see* Ex. 1002, ¶¶115–119; Ex. 1004, 6 (“Figure 3, the Z(N23T) mutant shows higher resistance to alkaline conditions than the Z domain when exposed to high pH values.”). That Linhult recognizes that additional mutations could further improve alkaline stability does not detract from Linhult’s teaching that a composition containing the single G29A mutation in SPA Domain B retains IgG binding. Ex. 1004, 6, *see id.* at 4 (“The Z-domain already possesses a significant tolerance to alkaline conditions.”).

d) Claims 2, 12, 15, and 25

Claims 2 and 12 depend from claim 1, and claims 15 and 25 depend from claim 14. We find Petitioner has shown by a preponderance of the evidence that the combination of Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims for the reasons stated in the Petition which we adopt as our own. *See* Pet. 25–28, 30.

We have reviewed Petitioner’s arguments and the underlying evidence cited in support and determine that Petitioner establishes that of Linhult, Abrahmsén, and Hober teaches the additional limitations of these dependent claims. Specifically, Petitioner asserts that Linhult describes multimerization of the ligand as recited in claims 2, 12, 15; and 25. Pet. 26 (citing Ex. 1004, 410; Ex. 1002 ¶¶112–114), *see id.* at 30 (citing Ex. 1002 ¶¶128–129).

Patent Owner does not offer arguments addressing Petitioner’s substantive showing with respect to claims 2, 12, 15, and 25. *See generally* PO Resp.

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6. *Summary*

For the foregoing reasons, we determine that Petitioner has shown by a preponderance of evidence that of claims 1–7, 10–20, and 23–26 of the ’765 patent are unpatentable based on the combination of Linhult, Abrahmsén, and Hober.

F. *Other Asserted Grounds*

Petitioner also asserts that claims 1–4, 12, 14–17, and 25 are unpatentable as obvious over Linhult and Abrahmsén (Pet. 18–30); claims 1–7, 10–20, and 23–26 are unpatentable as obvious over Linhult and Hober (*id.* at 30–48); claims 1–7, 10–20, 23–26 are unpatentable as obvious over Abrahmsén and Hober (*id.* at 49–60); claims 1–7, 10–20, and 23–26 are unpatentable over Berg and Linhult (’043 IPR Pet. 20–33); claims 2, 3, 15, and 16 are unpatentable over Berg, Linhult, and Hober (*id.* at 33–38); claims 1, 2, 5–7, 10–15, 18–20, and 23–26 are unpatentable over Berg and Abrahmsén (*id.* at 38–44); and claims 2–4, 15–17 are unpatentable over Berg, Abrahmsén, and Hober (*id.* at 44–46) under 35 U.S.C. §103(a). However, because Petitioner has already shown that the challenged claims 1–7, 10–20, and 23–26 are unpatentable over Linhult, Abrahmsén, and Hober as obvious, as discussed *supra*, we do not reach these additional asserted grounds. *See Beloit Corp. v. Valmet Oy*, 742 F.2d 1421, 1423 (Fed. Cir. 1984) (“The Commission . . . is at perfect liberty to reach a ‘no violation’ determination on a single dispositive issue.”); *Boston Sci. Scimed, Inc. v. Cook Grp., Inc.*, 809 F. App’x 984, 990 (Fed. Cir. 2020) (recognizing that “[t]he Board has the discretion to decline to decide additional instituted grounds once the petitioner has prevailed on all its challenged claims”).

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III. CONCLUSION¹⁴

For the foregoing reasons, we determine that Petitioner has demonstrated by a preponderance of the evidence that claims 1–7, 10–20, and 23–26 of the '765 patent are unpatentable on the bases set forth in the following table.

In summary:

Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–4, 12, 14–17, 25	103(a)	Linhult, Abrahmsén ¹⁵		
1–7, 10–20, 23–26	103(a)	Linhult, Hober ¹⁶		
1–7, 10–20, 23–26	103(a)	Linhult, Abrahmsén, Hober	1–7, 10–20, 23–26	
1–7, 10–20, 23–26	103(a)	Abrahmsén, Hober ¹⁷		

¹⁴ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this decision, we draw Patent Owner's attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. See 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. See 37 C.F.R. § 42.8(a)(3), (b)(2).

¹⁵ As explained above, we do not reach this '036 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

¹⁶ See *supra*, n.15.

¹⁷ See *supra*, n.15.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1-7, 10-20, 23-26	103(a)	Berg, Linhult ¹⁸		
2, 3, 15, 16	103(a)	Berg, Linhult, Hober ¹⁹		
1, 2, 5-7, 10-15, 18-20, 23-26	103(a)	Berg, Abrahmsén ²⁰		
2-4, 15-17	103(a)	Berg, Abrahmsén, Hober ²¹		
Overall Outcome			1-7, 10-20, 23-26	

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the preponderance of the evidence of record has shown that claims 1-7, 10-20, and 23-26 of the '765 patent are found unpatentable; and

FURTHER ORDERED because this is a final written decision, the parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

¹⁸ As explained above, we do not reach this '043 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

¹⁹ See *supra*, n.18.

²⁰ See *supra*, n.18.

²¹ See *supra*, n.18.

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Paper 43
Date: May 18, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

JSR CORPORATION and JSR LIFE SCIENCES, LLC,
Petitioner,

v.

CYTIVA BIOPROCESS R&D AB,
Patent Owner.

IPR2022-00041
IPR2022-00044
Patent 10,343,142 B2

Before ULRIKE W. JENKS, SHERIDAN K. SNEDDEN, and
SUSAN L. C. MITCHELL, *Administrative Patent Judges*.

JENKS, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining Some Challenged Claims Unpatentable
35 U.S.C. § 318

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Patent 10,343,142 B2

I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review of claims 1–7, 10–20, and 23–30 (“the challenged claims”) of U.S. Patent No. 10,343,142 B2 (Ex. 1001, “the ’142 patent”). We have jurisdiction under 35 U.S.C. § 6, and enter this Final Written Decision pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons set forth below, we determine that JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) has shown, by a preponderance of the evidence, that some of the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e).

A. *Consolidated Proceedings*

The two captioned proceedings (IPR2022-00041 and IPR2022-00044 (or “the ’044 IPR”)) involve the ’142 patent and challenge the same set of claims. The asserted grounds and prior art contentions are different in each proceeding. Consolidation is appropriate where, as here, the Board can more efficiently handle the common issues and evidence, and also remain consistent across proceedings. Under 35 U.S.C. § 315(d), the Director may determine the manner in which these pending proceedings may proceed, including “providing for stay, transfer, consolidation, or termination of any such matter or proceeding.” *See also* 37 C.F.R. § 42.4(a) (“The Board institutes the trial on behalf of the Director.”). There is no specific Board rule that governs consolidation of cases. Rule 42.5(a), however, allows the Board to determine a proper course of conduct in a proceeding for any situation not specifically covered by the rules and to enter non-final orders to administer the proceeding. *See* 37 C.F.R. § 42.5(a).

Therefore, on behalf of the Director under § 315(d), and for a more efficient administration of these proceedings, we consolidate IPR2022-

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00041 and IPR2022-00044 for purposes of rendering this Final Written Decision.

B. *Evidence*

Petitioner relies upon information that includes the following:

Ex. 1004, M. Linhult, *et al.*, *Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach*, 55 PROTEINS: STRUCTURE, FUNCTION, AND BIOINF., 407–16 (2004) (“Linhult”).

Ex. 1005, L. Abrahmsén, *et al.*, U.S. Patent No. 5,143,844 (issued Sept. 1, 1992) (“Abrahmsén”).

Ex. 1006, S. Hober, PCT Publication No. WO 03/080655 A1 (published Oct. 2, 2003) (“Hober”).

C. *Procedural History*

Petitioner filed a Petition for an *inter partes* review of the challenged claims under 35 U.S.C. § 311. Paper 1¹ (“Pet.”). Petitioner supported the Petition with the Declaration of Dr. Steven M. Cramer. Ex. 1002. Cytiva Bioprocess R&D AB (“Patent Owner”) filed a Patent Owner Preliminary Response to the Petition. Paper 8.

On May 19, 2022, pursuant to 35 U.S.C. § 314(a), we instituted trial (“Decision” or “Dec.” (Paper 9)) to determine whether any challenged claim of the ’142 patent is unpatentable.

¹ We note that the evidence filed in both proceedings is generally consistent in having the same exhibit number. Therefore, we reference exhibits and paper numbers as they appear in the record of IPR2022-00041, unless otherwise noted.

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In IPR2022-00041, Petitioner asserts the following grounds of unpatentability (Pet. 4):

Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–4, 12, 14–17, 25, 27–30	103(a)	Linhult, Abrahmsén
1–7, 10–20, 23–30	103(a)	Linhult, Hober
1–7, 10–20, 23–30	103(a)	Linhult, Abrahmsén, Hober
1–7, 10–20, 23–30	103(a)	Abrahmsén, Hober

In IPR2022-00044, Petitioner asserts the following grounds of unpatentability ('044 IPR Pet. 4):

Claim(s) Challenged	35 U.S.C. §	Reference(s)/Basis
1–7, 10–20, 23–26	103(a)	Berg, Linhult
2, 3, 15, 16	103(a)	Berg, Linhult, Hober
1, 2, 5–7, 10–15, 18–20, 23–26	103(a)	Berg, Abrahmsén
2–4, 15–17, 27–30	103(a)	Berg, Abrahmsén, Hober

Patent Owner filed a Patent Owner Response to the Petition. Paper 15 (“PO Resp.”). Patent Owner supported the Response with the Declaration of Dr. Daniel Bracewell (Ex. 2025). *See* PO Resp., iv (Exhibit List). Petitioner filed a Reply to the Patent Owner Response. Paper 28 (“Reply”). Petitioner

² The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the ’142 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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supported the Reply with a Reply Declaration from Dr. Steven M. Cramer. Ex. 1061. Patent Owner filed a Sur-reply to Petitioner's Reply. Paper 34 ("Sur-reply").

On February 16, 2023, the parties presented arguments at an oral hearing. Paper 35. The hearing transcript has been entered in the record. Paper 39 ("Tr.").

For the reasons set forth below, we determine that Petitioner has shown by a preponderance of the evidence that claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the '142 patent are unpatentable, but find that Petitioner has not shown by a preponderance of the evidence that claims 4 and 17 are unpatentable.

D. *Real Parties in Interest*

Petitioner identifies itself, JSR Corporation and JSR Life Sciences, LLC, along with JSR Micro NV, as the real parties-in-interest. Pet. 2. Patent Owner identifies itself, Cytiva Bioprocess R&D AB, along with Cytiva Sweden AB and Danaher Corporation as real parties-in-interest. Paper 5, 1.

E. *Related Matters*

The '142 patent is at issue in *Cytiva BioProcess R&D et al. v. JSR Corp. et al.*, Civil Action No. 21-310-RGA (D. Del.). Pet. 2; Paper 5, 1.

In addition to the '142 patent challenged here, Petitioner has filed Petitions for *inter partes* review of related U.S. patents as follows: U.S. Patent No. 10,213,765 B2 ("the '765 patent") in IPR2022-00036 and IPR2022-00043; and U.S. Patent No. 10,875,007 B2 ("the '007 patent") in IPR2022-00042 and IPR2022-00045. Pet. 2–3; Paper 5, 1–2. Petitioner indicates that the '765 patent and the '007 patent are also being asserted in

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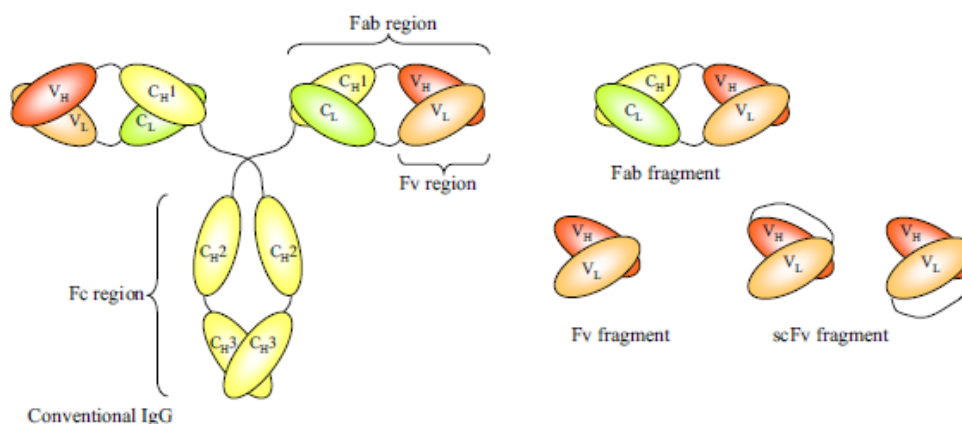
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the above-cited district court case. Pet. 3. The parties further list a pending application in the same family, U.S. App. Serial No. 17/107,600. Pet. 2; Paper 5, 2.

F. *Subject matter background*

Antibodies (also called immunoglobulins) are glycoproteins, which specifically recognize foreign molecules. These recognized foreign molecules are called antigens. Ex. 2001, 1. A schematic representation of the structure of a conventional IgG and fragments is shown below:



The figure (Ex. 2001, 2 (Fig. 1)), reproduced above, shows

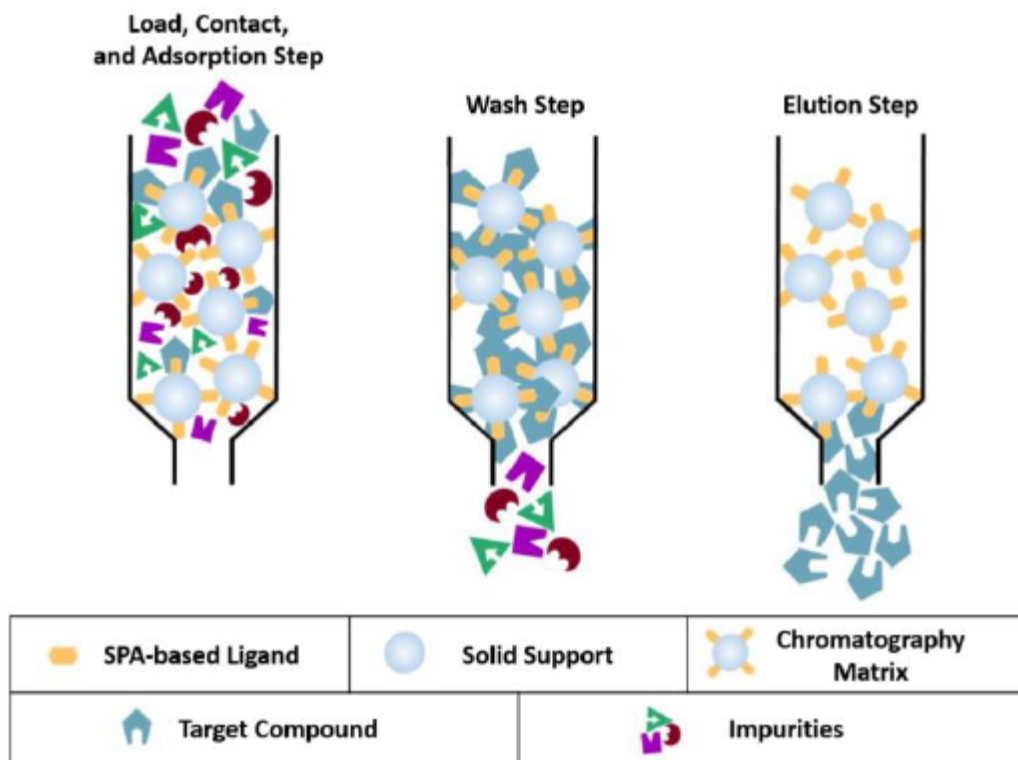
the structure of a conventional IgG and fragments that can be generated thereof. The constant heavy-chain domains CH1, CH2 and CH3 are shown in yellow, the constant light-chain domain (CL) in green and the variable heavy-chain (VH) or light-chain (VL) domains in red and orange, respectively. The antigen binding domains of a conventional antibody are Fabs and Fv fragments. Fab fragments can be generated by papain digestion. Fvs are the smallest fragments with an intact antigen-binding domain. They can be generated by enzymatic approaches or expression of the relevant gene fragments (the recombinant version). In the recombinant single-chain Fv fragment, the variable domains are joined by a peptide linker. Both possible configurations of the variable domains are shown, i.e. the carboxyl terminus of VH fused to the N-terminus of VL and vice

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versa.

Ex. 2001, 2; *see also* PO Resp. 5.

Below is a generic, exemplary schematic that shows how affinity purification typically works:



The figure shows the schematic of the loading, contact, and adsorbing step onto a column, followed by the wash step, and finally the elution and collection of the target compound. Ex. 1002 ¶ 24 (citing Ex. 1014 §§ 1.1, 4.2.); *see also* PO Resp. 7 (“In a typical process, the composition containing the desired antibody then is loaded onto (i.e., pumped or injected into) the column.”); Pet. 6; *see generally* Ex. 1014.

G. *The '142 patent (Ex. 1001)*

The '142 patent is titled “Chromatography Ligand Comprising Domain C from *Staphylococcus Aureus* Protein A for Antibody Isolation.”

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Ex. 1001, (54). The '142 patent relates to an affinity ligand that is used for antibody isolation. *Id.* at 1:39–41. The '142 patent explains that chromatography is used in large-scale economic production of drugs and diagnostics in which proteins are produced by cell culture and then separated from the mixture of compounds and other cellular components to a sufficient purity. *Id.* at 1:52–61. One type of chromatography matrix for this purifying process includes immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG). *Id.* at 2:4–13. The '142 patent further explains that “[a]s in all process technology, an important aim is to keep the production costs low” by reusing matrices via cleaning protocols such as an alkaline protocol known as Cleaning In Place (CIP). *Id.* at 2:14–29. However, harsh treatments may impair the chromatography matrix materials such that there is a need for stability towards alkaline conditions for an engineered protein ligand. *Id.* at 2:31–48.

The '142 patent discloses that Protein A, known as SpA, is a constituent of the cell wall of the bacterium *Staphylococcus aureus*, and is widely used as a ligand in affinity chromatography matrices due to its ability to bind with IgG. *Id.* at 2:49–54. SpA is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind to antibodies at the Fc region, and it has been shown that each of these domains binds to certain antibodies at the Fab region. *Id.* at 2:54–63.

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Domain C from SpA is defined by SEQ ID NO: 1 and is reproduced below.

```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1           5           10           15
Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
           20           25           30
Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
           35           40           45
Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
           50           55

```

Id. at 4:26 (highlighting added by Board); 15:1–38; *see also* Pet. 9. SEQ ID NO: 1 shows domain C has Glycine (Gly) as an amino acid at the position 29 as annotated via red highlighting. According to the '142 patent, it has already been shown “that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A” and the '142 patent discloses that from experiments, “the present inventors have quite surprisingly shown that the SpA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product.” Ex. 1001, 5:38–40, 51–55. The '142 patent discloses, “it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences -asparagine-glycine- and -asparagine-serine.” *Id.* at 5:62–66. The '142 patent then discloses “[q]uite surprisingly, the Domain C ligand of the invention presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29” and “[t]hus, in a specific embodiment, the chromatography ligand according to the invention comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A.” *Id.* at 5:67–6:3, 6:49–52. The '142 patent discloses that a multimeric chromatography ligand

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(also denoted a “multimer”) can be comprised of at least two domain C units and that a chromatography matrix can be comprised of ligands coupled to an insoluble carrier. *Id.* at 7:27–29, 8:21–23. The ’142 patent discloses a column study of alkaline stability of its Protein A-derived ligands and a testing of the Fab-binding of its ligands. *Id.* at 10:32–14:59. The study includes using an injection liquid and solution along with human normal immunoglobulin as a target compound in chromatography experiments, ligand coupling and column packing, adsorbance measurements, washing out unbound samples, and eluting bound material. *Id.* at 11:11–18, 12:25–34, 13:32–37.

1. Illustrative Claim

Claims 1 and 14 are the independent claims challenged by Petitioner in this proceeding. Independent claim 1, reproduced below, is illustrative of the subject matter:

1. A process for isolating one or more target compound(s), the process comprising:

(a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:

(i) a solid support; and

(ii) at least one ligand coupled to the solid support, the ligand comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1; and

(b) adsorbing the target compound(s) to the ligand; and,

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(c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand.

Ex. 1001, 15:40–56. Claim 14 is similar to claim 1 but recites “a ligand” rather than “at least one ligand,” and wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids “in alignment with SEQ ID NO. 1” rather than “contiguous amino acids of a modified SEQ ID NO. 1.” *Id.* at 16:57–17:6.

II. ANALYSIS

A. *Principles of Law*

“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify “with particularity . . . the evidence that supports the grounds for the challenge to each claim”)). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner must demonstrate by a preponderance of the evidence³ that the claims are unpatentable. 35 U.S.C. § 316(e); 37 C.F.R. § 42.1(d). A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter,

³ The burden of showing something by a preponderance of the evidence requires the trier of fact to believe that the existence of a fact is more probable than its nonexistence before the trier of fact may find in favor of the party who carries the burden. *Concrete Pipe & Prods. of Cal., Inc. v. Constr. Laborers Pension Tr. for S. Cal.*, 508 U.S. 602, 622 (1993).

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as a whole, would have been obvious at the time of the invention to a person having ordinary skill in the art. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations, including “the scope and content of the prior art”; “differences between the prior art and the claims at issue”; “the level of ordinary skill in the art;” and any “objective evidence of non-obviousness.” *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. *Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the

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technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner asserts that a person of ordinary skill in the art would have had

(1) at least an advanced degree (*e.g.*, a Master’s or Ph.D.) in biochemistry, process chemistry, protein chemistry, chemical engineering, molecular and structural biology, biochemical engineering, or similar disciplines; (2) several years of post-graduate training or related experience (including industry experience) in one or more of these areas; and (3) an understanding of the various factors involved in purifying proteins using chromatography.[] Such a person would have had multiple years of experience with affinity ligand design and protein purification.

Pet. 9–10 (citing Ex. 1002 ¶¶ 13–14). Patent Owner does not dispute Petitioner’s definition of the person of ordinary skill. *See generally* PO Resp. Because Petitioner’s proposed definition is unopposed and appears consistent with the Specification and art of record, we apply it here.

C. *Claim Construction*

The Board applies the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.200(b) (2021). Under that standard, claim terms “are generally given their ordinary and customary meaning” as understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc).

Petitioner contends that based on Patent Owner’s implicit construction in the district court litigation “that the term ‘the ligand comprising at least

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two polypeptides’ refers to a multimeric ligand (such as a tetramer) comprised of multiple polypeptides, each of which is a monomer.” Pet. 15 (citing Ex. 1020 ¶¶ 41, 50, 58, 62, 74, 87).

Patent Owner does not contest Petitioner’s construction. *See generally* PO Resp.

According to the Specification, “the present invention . . . relates to a multimeric chromatography ligand (also denoted a ‘multimer’) comprised of at least two Domain C units, or a functional fragments or variants thereof.” Ex. 1001, 7:27–30. The Specification additionally recites that a multimer containing only domain C units can, however, include linkers. *Id.* 7:39–41. In addition, the Specification describes that “the multimer comprises one or more additional units, which are different from Domain C.” *Id.* 7: 44–45. Based on these disclosures in the Specification, a multimer is composed of at least two or more monomers.

Because Petitioner’s construction is consistent with the ’142 patent’s express construction of the term, and because Patent Owner agrees with Petitioner’s construction, we apply it here.

D. *Overview of Asserted References*

1. *Linhult (Ex. 1004)*

Linhult is titled “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach.” Ex. 1004, 1. Linhult discloses that due to the high affinity and selectivity of Staphylococcal protein A (SPA), “it has a widespread use as an affinity ligand for capture and purification of antibodies,” but that “it is desirable to further improve the stability in order to enable an SPA-based affinity medium to withstand even longer exposure to the harsh conditions

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associated with cleaning-in-place (CIP) procedures.” *Id.*, Abstr. Linhult discloses, “[t]o further increase the alkaline tolerance of SPA, we chose to work with Z, which is a small protein derived from the B domain of SPA.” *Id.* at 2.

Figures 1A and 1B of Linhult are reproduced below.

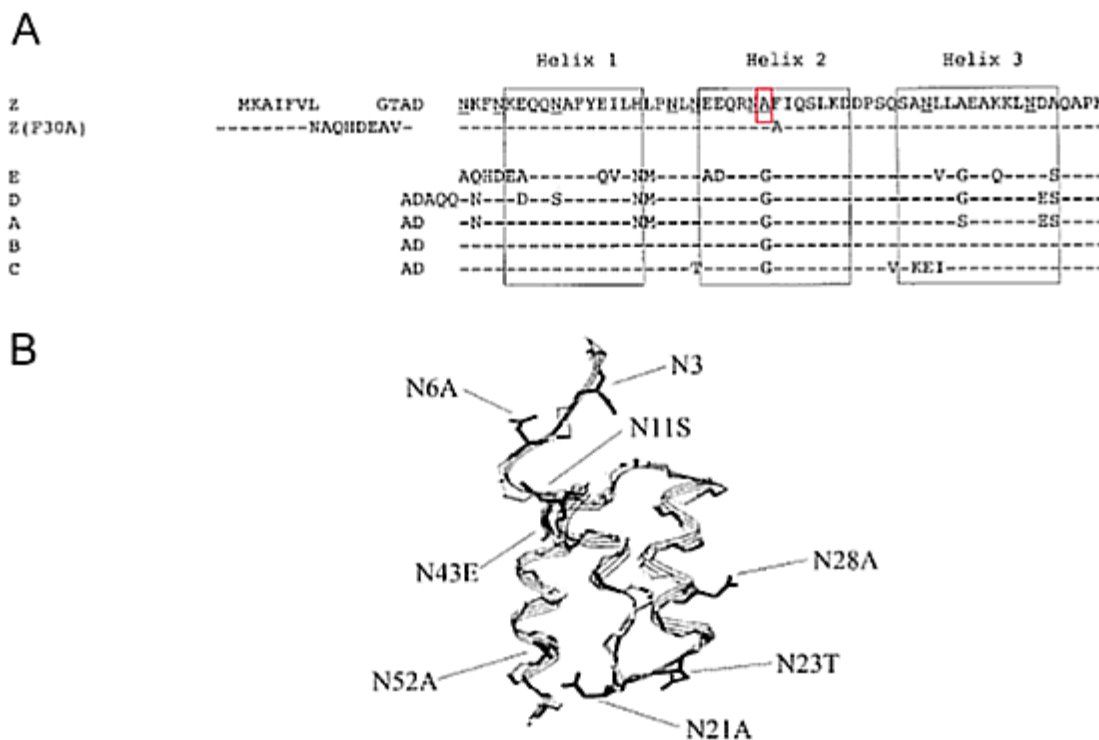


Figure 1A shows “[a]mino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C)” in which the horizontal lines indicate amino acid identity and “one glycine in the B domain [is] replaced [and] underlined” as annotated by the Board with a red box. *Id.* “Z(F30A), and all mutants thereof includes the same N-terminal as Z(F30A)” and

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“Z(N23T) was constructed with the same N-terminal as Z.” *Id.*⁴ Figure 1B shows “[t]he three-dimensional structure of the Z domain” and “the different substitutions are indicated.” *Id.* Specifically, Linhult discloses,

[t]he B domain has been mutated in order to achieve a purification domain resistant to cleavage by hydroxylamine. An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine.[] Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.[] Protein Z is well characterized and extensively used as both ligand and fusion partner in a variety of affinity chromatography systems.

Id. Using a 0.5 M NaOH cleaning agent and “a total exposure time of 7.5 h for Z(F30A) and mutants thereof,” Linhult determines that “N23 seems to be very important for the functional stability after alkaline treatment of Z(F30A)” and “Z(F30A, N23T) shows only a 28% decrease in capacity despite the destabilizing F30A-mutation.” *Id.* at 410–11; Figs. 2, 3. Linhult reports that “[h]ence, the Z(F30A, N23T) is almost as tolerant as Z and is thereby the most improved variant with Z(F30A) as scaffold.” *Id.* at 411; Figs. 2, 3.

Linhult further discloses that “Z, Z(F30A), and mutated variants were covalently coupled to HiTrap™ affinity columns,” that “[t]he Z domain includes 8 asparagines (N3, N6, N11, N21, N23, N28, N43, and N52; Fig. 1),” and that “since the amino acid is located outside the structured part of the domain, it will most likely be easily replaceable during a multimerization of the domain to achieve a protein A–like molecule.” *Id.* at 410. Linhult

⁴ The mutation N23T having a change in amino acid correlates with the amino acid N next to the “Helix 2” box of Figure 1A as annotated by Petitioner. *See* Pet. 12.

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confirms that “the affinity between Z(F30A) and IgG was retained despite the mutation.” *Id.* In Linhult’s studies, “[h]uman polyclonal IgG in TST was prepared and injected onto the columns in excess” and “[a] standard affinity chromatography protocol was followed.” *Id.*

2. *Abrahmsén (Ex. 1005)*

Abrahmsén “relates to a recombinant DNA fragment coding for an immunoglobulin G ([I]gG) binding domain related to staphylococcal protein A . . . and to a process for cleavage of a fused protein expressed by using such fragment or sequence.” Ex. 1005, 1:8–13. Abrahmsén discloses that “[b]y making a gene fusion to staphylococcal protein A any gene product can be purified as a fusion protein to protein A and can thus be purified in a single step using IgG affinity chromatography.” *Id.* at 1:22–26. Abrahmsén explains that Protein A has “5 Asn-Gly in the IgG binding region of protein A” which “makes the second passage through the column irrelevant as the protein A pieces released from the cleavage will not bind to the IgG.” *Id.* at 1:58–63. Abrahmsén provides a solution to this problem “by adapting an IgG binding domain so that no Met and optionally no Asn-Gly is present in the sequence.” *Id.* at 1:64–67.

Abrahmsén discloses that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén discloses that the recombinant DNA fragment can “cod[e] for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the

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Asn-Gly coding constellation has been replaced by an alanine codon.” *Id.* at 2:32–37. According to Abrahmsén, from a simulation of the Gly to Ala amino acid change in the computer, it was “concluded that this change would not interfere with folding to protein A or binding to IgG.” *Id.* at 5:13–16.

3. *Hober (Ex. 1006)*

Hober “relates to . . . a mutant protein that exhibits improved stability compared to the parental molecule” and “also relates to an affinity separation matrix, wherein a mutant protein according to the invention is used as an affinity ligand.” Ex. 1006, 1. Hober discloses that removal of contaminants from the separation matrix involves “a procedure known as cleaning-in-place (CIP)” but “[f]or many affinity chromatography matrices containing proteinaceous affinity ligands,” the alkaline environment “is a very harsh condition and consequently results in decreased capacities owing to instability of the ligand.” *Id.* at 1–2. According to Hober, stability to alkaline conditions can be engineered into a protein. *Id.* at 2. To improve the stability of a Streptococcal albumin-binding domain (ABD) in alkaline environments, it has been reported to involve the role of peptide conformation in the rate and mechanism of deamidation of asparaginyl residues and that “the shortest deamidation half time have been associated with the sequences -asparagine-glycine and -asparagine-serine.” *Id.* at 2. Further, from a study of a mutant of ABD that was created, it was concluded that “all four asparagine residues can be replaced without any significant effect on structure and function.” *Id.* at 2–3. Hober points out that the staphylococcal protein A (SPA) contains domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species and

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reagents of this protein with their high affinity and selectivity have found a widespread use in the field of biotechnology. *Id.* at 3. Accordingly, “there is a need in this field to obtain protein ligands capable of binding immunoglobulins, especially via the Fc-fragments thereof, which are also tolerant to one or more cleaning procedures using alkaline agents.” *Id.* at 4.

In one embodiment of Hober, a multimer “comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A” in which “asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids” for advantageous structural stability reasons wherein “the glycine residue in position 29 of SEQ ID NO: 1 has also been mutated, preferably to, an alanine residue.” *Id.* at 12. Hober’s SEQ ID NO: 1 and is reproduced below.

Ala	Asp	Asn	Lys	Phe	Asn	Lys	Glu	Gln	Gln	Asn	Ala	Phe	Tyr	Glu	Ile
1				5					10					15	
Leu	His	Leu	Pro	Asn	Leu	Asn	Glu	Glu	Gln	Arg	Asn	Gly	Phe	Ile	Gln
			20					25					30		
Ser	Leu	Lys	Asp	Asp	Pro	Ser	Gln	Ser	Ala	Asn	Leu	Leu	Ala	Glu	Ala
		35					40					45			
Lys	Lys	Leu	Asn	Asp	Ala	Gln	Ala	Pro	Lys						
	50					55									

Id. at SEQUENCE LISTING 1. SEQ ID NO: 1 shows a domain of *Staphylococcus aureus* having Glycine (Gly) as an amino acid at the position 29, as annotated by the Board via red highlighting.

Hober further discloses that its matrix for affinity separation “comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which [in the] protein at least one asparagine residue has been mutated to an amino acid other than glutamine.” *Id.* at 13. For its method of isolating an immunoglobulin, Hober discloses “in a first step, a

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solution comprising the target compounds, . . . is passed over a separation matrix under conditions allowing adsorption of the target compound to ligands present on said matrix” and “[i]n a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption, i.e. release of the target compound.” *Id.* at 13.

E. *Obviousness in view of Linhult, Abrahmsén, and Hober*

1. *Petitioner’s Contentions*

a) *Claims 1 and 14*

Petitioner contends that “*Linhult* describes the common use of chromatography matrices in the biotechnology field, and, more specifically, SPA-based chromatography matrices to isolate target compounds.” Pet. 16 (citing Ex. 1002 ¶ 83). Petitioner contends that “*Linhult* describes a process whereby a ‘[h]uman polyclonal IgG in TST^[5] was prepared and injected onto the columns in excess,’ ‘[a] standard affinity chromatography protocol was followed,’ and ‘eluted material was detected.’” Pet. 17 (citing Ex. 1004, 4). Petitioner contends that a person of ordinary skill in the art “would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of loading a liquid comprising the target compound onto the column, thereby allowing the liquid to contact the recited SPA-based chromatography matrix.” *Id.* (citing Ex. 1002 ¶¶ 88–89). In other words, Petitioner contends that the contacting step is a well-known step in the field of affinity chromatography.

⁵ TST is a solution containing 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20. Ex. 1004, 4.

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Petitioner contends that “[a]dsorbing target compounds to SPA-based ligands coupled to the solid supports was a well-known and conventional feature of SPA-based affinity chromatography.” Pet. 23 (citing Ex. 1002 ¶ 119, *see also id.* ¶¶ 120–121; Ex. 1004, 4).

Petitioner contends that “a [person of ordinary skill in the art] would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of eluting target compounds from SPA-based ligands coupled to the solid supports in a chromatography matrix.” Pet. 24 (citing Ex. 1002 ¶ 126, *see also id.* ¶¶ 123–127; Ex. 1004 ¶ 4). Petitioner, therefore contends that it is well-known that a standard affinity chromatography protocol contains three active steps: (1) contacting, (2) adsorbing, and (3) eluting.

Petitioner contends that Linhult teaches a chromatography matrix. Pet. 18. Specifically, Linhult teaches using a HiTrap chromatography affinity column made up of agarose beads that serve as a solid support for “coupling SPA-based ligands.” Pet. 18 (citing Ex. 1004, 4; Ex. 1002 ¶ 95). “*Linhult* discloses that its SPA-based ligands were ‘coupled to’ the solid support agarose beads [] contained in HiTrap™ affinity columns.” *Id.* at 18 (citing Ex. 1004, 4). Petitioner contends that “Linhult discloses that ‘multimerization’ of SPA monomers is performed to ‘achieve’ an ‘[SPA-]like’ affinity ligand.” *Id.* at 19 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 100–103). Petitioner contends that “Figure 1(a), *Linhult* describes at least 55 amino acids of SPA’s naturally-occurring C domain (i.e., SEQ ID NO. 1).” *Id.* (citing Ex. 1004, 1, Fig. 1(a); *see* Ex. 1005, Fig. 2; Ex. 1006, Fig. 1; Ex. 1008, 639, Fig. 1). Petitioner contends “that all ‘five SPA domains show individual affinity for the Fc-fragment . . . as well as certain Fab-fragments

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of [antibodies] from most mammalian species.” *Id.* at 20 (citing Ex. 1004, 1).

Petitioner contends that it was “known that individual SPA domains, including the C domain, could be used to construct SPA-based affinity ligands for purifying proteins.” *Id.* at 20 (citing Ex. 1002 ¶¶ 29, 101; Ex. 1004, 1; Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). Petitioner contends that Linhult teaches a person of ordinary skill in the art “that avoiding the Asn₂₈-Gly₂₉ dipeptide sequence through a G29A mutation, including on the C domain, would yield an SPA-based ligand having increased alkali-stability.” *Id.* at 21 (citing 1002 ¶¶ 109–113; Ex. 1011; Ex. 1012; Ex. 1013). Petitioner acknowledges that “*Linhult* does not expressly disclose a C(G29A)-based SPA ligand,” but asserts that “[r]egardless, it would have been obvious to a [person of ordinary skill in the art] to modify *Linhult* based on the teachings of *Abrahmsén* to incorporate a C(G29A)-based SPA ligand in a chromatography matrix.” *Id.* at 23 (Ex. 1002 ¶¶ 108–117). Petitioner contends “*Abrahmsén* expressly discloses ‘a recombinant DNA coding for **any of** the E D A B C domains of [SPA], wherein the glycine codon(s) in the Asn_[28]-Gly_[29] coding constellation has been replaced by an alanine codon.’” *Id.* at 22 (bracketing in and emphasis in original) (citing Ex. 1005, 2:32–37).

A [person of ordinary skill in the art] would have had good reason to combine the teachings from *Abrahmsén* with *Linhult* because a G29A mutation was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence, i.e., the “most sensitive amino acid sequence to alkaline conditions,” such as those used in CIP. (Ex. 1002 ¶¶ 116, 129; Ex. 1004, [2].) Moreover, a [person of ordinary skill in the art] would have been drawn to a C-domain-based ligand, which, as *Linhult* describes, shows individual affinity for antibodies and

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already includes 23T (as well as 43E), which it disclosed as providing “remarkably increased” stability. (Ex. 1002 ¶¶112-13; Ex. 1004, [1], [8–9].)

Pet. 22–23.

[A]pplying the teachings of *Abrahmsén* with *Linhult* would have involved merely combining known elements in the field (e.g., a process for isolating one or more target compounds using an affinity chromatography matrix comprising a G29A-containing ligand coupled to a solid support, as in *Linhult*, and a C(G29A)-based amino acid sequence, as in *Abrahmsén*) according to known ligand-construction methods to yield a predictable results (e.g., a process for isolating one or more target compounds using the recited affinity chromatography matrix). (Ex. 1002 ¶130.) See, e.g., *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 415-21 (2007); *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1239-40 (Fed. Cir. 2010).

Pet. 24–25. Petitioner further contends “*Hober*’s disclosure is in the context of SPA-based affinity chromatography utilizing G29A-containing ligands, and, in fact, further confirms that the teachings of *Abrahmsén* are applicable in this context.” Pet. 48 (citing Ex. 1002 ¶¶ 236–250; Ex. 1006, 10–12); Ex. 1006, 12 (“the present multimer also comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A. . . . for structural stability reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to an alanine residue”).

b) Claims 4 and 17

Petitioner contends that “[t]he capability of “bind[ing] to the Fab part of an antibody,” as recited in claims 4 and 17, is an inherent property of the recited C(G29A)-based SPA ligand.” Pet. 28 (citing Ex. 1002 ¶¶ 141–148).

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c) Claims 2, 3, 12, 15, 16, 25, and 27–30

With respect to claims 2, 3, 12, 15, 16, 25, and 27–30, Petitioner directs our attention to where in the asserted art of record the various limitations of the dependent claims may be found. *See* Pet. 26–28, 30–31.

2. Patent Owner’s Contentions

Patent Owner argues that the Petition fails to demonstrate that there would have been motivation and a reason to make and use the chromatography matrix as claimed (PO Resp. 17–38) for the following reasons: that the Petition has not established that there is a reasonable expectation of success in arriving at the claimed matrix (*id.* at 48–54); that the art teaches away from making the G29A modification (*id.* at 38–44); and that the artisan would have been motivated to make alternative mutations than the claimed ones (*id.* at 44–48).

a) Matrix

According to Patent Owner, “Petitioners fail to explain *why* the POSA would have been motivated to select Domain C’s amino acid sequence as the foundation for an engineered SPA ligand with favorable properties.”

PO Resp. 18. Specifically arguing that the obviousness analysis requires the prior art be viewed as a whole. PO Resp. 20 (citing *In re Wesslau*, 353 F.2d 238 (CCPA 1965); *In re Enhanced Sec. Rsch., LLC*, 739 F.3d 1347, 1355 (Fed. Cir. 2014); *Impax Lab’ys Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018)). In other words, because obviousness requires considering the prior art as a whole and no one was working on domain C at the time of the invention, Patent Owner asserts, it would not have been obvious to select domain C for further development or genetic modification.

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Patent Owner argues that because nobody was working on domain C at the time the invention was filed, the selection of domain C for further development could not possibly be obvious. *See* PO Resp. 23 (“Reliance on *KSR* also is foreclosed by the evidence that no one in the art was seeking to modify Domain C.”), *see also id.* at 24 (“But no prior art cited by Petitioners singles Domain C out for further development. Ex. 2025 ¶¶ 89–96”), *id.* at 26–27 (“Dr. Cramer [Petitioner’s expert] himself highlights, it would have been natural for the POSA to further develop the domain—Domain B—that was best understood and for which there was a crystal structure available. Ex. 1002 ¶ 33; Ex. 2015 at 137:20–138:19; Ex. 2017”), *id.* at 28 (“The notion that this body of work would lead the POSA to discard the improved ligands the references themselves focus on, and instead start experimenting with mutations to Domain C—strains credulity. Ex. 2025 ¶¶ 92–95”).

According to Patent Owner, neither Linhult nor Abrahmsén supply the motivation to start with domain C. “Linhult focuses exclusively on, and concerns improvements to, the alkaline stability of Domain Z by mutating asparagine residues. *See* Ex. 2025 ¶¶ 64–67, 92.” PO Resp. 29. “Rather than use Domain C, the POSA reviewing Linhult would be motivated to keep working with Domain Z, adopting the N23T mutation. Ex. 2025 ¶ 67 & n.3.” PO Resp. 30. “Neither Abrahmsén itself nor the Petition provide[s] any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domains. *WBIP[LLC v. Kohler Co.]*, 829 F.3d 1317, 1337 (Fed. Cir. 2016)].” PO Resp. 31.

b) Reasonable Expectation of Success

Patent Owner argues that “the field of protein engineering is notoriously unpredictable.” PO Resp. 22 (citing Ex. 2025 ¶¶ 50–52).

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Arguing that “despite their supposed structural similarity, there are a number of differences between the naturally-occurring domains of protein A, including five different amino acids in the sequences of Domain B (with which the industry was quite familiar) and Domain C (which remained virtually ignored as of the priority date).” *Id.* at 22–23 (citing Ex. 2025 ¶ 48).

Protein engineering is a highly complex and unpredictable field and was all the more so as of the priority date more than fifteen years ago. *See, e.g.,* Ex. 2025 ¶¶ 50-52. . . . As amply demonstrated by the effect of the G29A mutation on Domain Z’s Fab-binding ability, even a single amino acid substitution can drastically alter the properties of a protein. Ex. 2025 ¶ 52; Ex. 2015 at 51:15-52:1 ([Dr. Cramer, Petitioner’s expert] agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability), 18:10-12, 73:16-20.

PO Resp. 35–36.

Patent Owner argues that

The Federal Circuit has rejected arguments premised on the notion that a homologous structure renders an invention obvious, particularly given the difficulty and uncertainty in the art as of the priority date. *See, e.g., Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208 (Fed. Cir. 1991) (holding the use of a monkey gene to probe for a roughly 90 percent “homologous” human gene would not have been obvious, particularly given expert testimony that isolating a particular gene would have been “difficult” and the lack of certainty in the endeavor).

PO Resp. 37. Specifically, Patent Owner argues that the Fab-binding capability of a ligand could not have been predicted, and therefore, there is no reasonable expectation of success in using the claimed ligand in a process of purifying a target compound. *See* PO Resp. 48–53.

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c) Teaching Away

Patent Owner argues that the prior art would have told the person of ordinary skill in the art to avoid a G29A a modification to domain C.

PO Resp. 39. In other words, its Patent Owner's contention that the prior art teaches away from making this modification. "The very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding 'negligible' when implemented in Domain B." PO Resp. 41. Patent Owner argues that a person seeking to improve Fab binding would have avoided a G29A substitution of domain C. PO Resp. 41–44.

d) Additional Modifications

Patent Owner argues that "the prior art would have taught the POSA to make asparagine substitutions, not glycine substitutions, to address alkaline stability concerns." PO Resp. 44. In other words, Patent Owner argues the prior art would have suggested making additional substitutions most notably in the asparagine residues of domain C. *Id.* at 45.

3. Petitioner's Reply

In response, Petitioner argues that

Abrahmsén and *Hober* each expressly pointed to a C(G29A) mutation (Ex. 1005, 2:32-37; Ex. 1006, 12), which was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence (*see, e.g.*, Ex. 1004, [2]). As the Board recognized, "*Abrahmsén* provides motivation for making [the G29A] mutation in **any of the IgG binding domains.**" (Decision, 24; *see also* Ex. 1057, 97:3-16 (Dr. Bracewell admitting that *Abrahmsén* discloses a G29A mutation to any of the five domains, including Domain C).)

Reply 2.

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A POSA would have reasonably expected success in combining these teachings to achieve the claimed affinity chromatography matrix given the well-known fact that each individual domain, including Domain C, has affinity for antibodies (Ex. 1004, [1]), as well as *Abrahmsén*'s confirmation that G29A “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1057, 99:13-101:21 Ex. 1005, 5:13-16; Ex. 1002 ¶131).

Id. at 3.

Petitioner argues that Patent Owner “has not disputed that *Abrahmsén* disclosed that G29A ‘would not interfere with folding to protein A or binding to IgG.’ (Ex. 1005, 2:32–37, 5:4–16; Ex. 1057, 109:20–110:17.) Nor does it take issue with its own statements in *Hober* that G29A is advantageous for ‘structural stability reasons.’ (Ex. 1006, 12.)” *Id.* at 9. Petitioner contends that Patent Owner’s lack of binding argument is contradicted by “*Abrahmsén* and *Hober*, which make clear that G29A does not affect the ability of an SPA ligand to bind to an antibody. (Ex. 1005, 2:32–37, 5:4–16; Ex. 1006, 12.)” *Id.* at 10.

Petitioner argues that “a POSA would have started with any one of the naturally occurring domains. (Decision, 26-28.) To then increase alkali stability, a POSA would have made the simplest, well-known substitution: G29A. (Section II.A.1–2; Ex. 1061 ¶¶8–15.)” Reply 11.

Petitioner argues “that Fab-binding was an inherent feature of a C(G29A)-based ligand—which [Patent Owner] does not appear to dispute. (Decision, 34; [Prelim.] Resp., 53–54.) In fact, [Patent Owner] acknowledges that ‘C(G29A)-based SPA ligands retained substantial Fab-binding ability.’ (Resp., 56–57.)” *Id.* at 14.

Petitioner argues that “Fab-binding is not being used [in the Petition] as part of a finding of a motivation to combine; rather, it is an inherent

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property [of the composition] being claimed. And necessarily present properties do not add patentable weight when they are claimed as limitations. *In re Kubin*, 561 F.3d 1351, 1357 (Fed. Cir. 2009).” Reply 15–16. Petitioner further argues that Patent Owner’s reliance on *Honeywell* is misplaced because “*Honeywell* had to do with an inherent property being used as a teaching in an obviousness analysis; it did not involve a limitation in the challenged claim reciting an inherent property.” Reply 15 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. De C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017); *see also Pernix Ireland Pain v. Alvogen Malta Operations*, 323 F. Supp. 3d 566, 607(D. Del. 2018)).

4. Patent Owner’s Sur-reply

Patent Owner argues that “Petitioners, and the Institution Decision, overlook an important point of consensus between the parties’ experts: the field of protein engineering is notoriously *unpredictable*.” Sur-reply 2. Patent Owner maintains that Petitioner has not identified a motivation to start from domain C. *Id.* at 3. Patent Owner argues that “Petitioners would have the Board look past the multitude of references teaching a preference for Domains B and Z—including Petitioners’ foundational references—and seize upon fleeting mentions of Domain C.” *Id.* at 7 (citing *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965)”). Patent Owner argues that “[m]ere sequence homology does not make the field predictable, as both experts observe, Ex. 2015 at 51:15-52:1, 56:4-12, 75:12-22, 73:16-20; Ex. 2025 ¶¶ 50-52; Ex. 2049 at 72:1-73:12, and as the vastly different Fab-binding properties of the near-identical Domains B and Z well illustrate, Ex. 2029 at 8.” *Id.* at 8–9.

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Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7–10.” Sur-reply 11.

5. *Analysis*

a) Independent Claims 1 and 14

Claims 1 and 14 of the ’142 patent are directed to a method of isolating a target compound using a chromatography matrix composition. The claims recite three active steps: (1) contacting, (2) adsorbing, and (3) eluting the target compound from the chromatography matrix. The claims further stipulate that the chromatography matrix composition (a solid support) has the following features: the ligand attached is attached to the matrix and the ligand is made up of at least two polypeptides comprising 55 contiguous amino acids of SEQ ID NO: 1⁶ having a G29A mutation. Claim 14 is similar to claim 1, except that claim 14 recites “at least 55 amino acids in alignment with SEQ ID NO. 1” instead of “at least 55 contiguous amino acids of modified SEQ ID NO. 1” as recited in claim 1.

(a) Process Steps

Petitioner asserts that the combination of Linhult, Abrahmsén, and Hober teaches or suggests the standard affinity chromatography process steps of (1) contacting, (2) adsorbing, and (3) eluting for the reasons set forth in the Petition. Pet. 16–31; Ex. 1002 ¶ 24.

⁶ Wild type amino acid sequence of domain C from *Staphylococcus* protein A (SPA). See Ex. 1001, 4:27, 6:35–36, 6:51–52.

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Linhult teaches making affinity chromatography columns with protein Z, Z(F30A), and other mutated variants. These modified proteins were covalently attached to HiTrap columns in Linhult using NHS-chemistry. Ex. 1004, 4. Linhult uses an affinity matrix column to isolate IgG and measures the loading capacity of the column after repeated cleaning in place (CIP) cycles. Ex. 1004, 4. In Linhult's studies, human polyclonal IgG was prepared and injected onto the columns in excess and "[a] standard affinity chromatography protocol was followed." *Id.* at 4. We find that Linhult's loading of IgG onto the column satisfies the contacting step as recited in the claims. Ex. 1004, 4, *see also id.* ("The columns were pulsed with TST (25mMTris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20) and 0.2 M HAc, pH 3.1. Human polyclonal IgG in TST was prepared and injected onto the columns in excess. A standard affinity chromatography protocol was followed for 16 cycles."). Linhult teaches that "the amount of eluted IgG was measured after each cycle to determine the total capacity of the column." *Id.* Linhult thereby expressly teaches the claimed contacting and eluting steps, and following standard chromatography protocols, the adsorbing step is implied. *Id.*; Ex. 1002 ¶ 24 ("After loading is completed, an additional step to wash out certain remaining impurities is employed. ([Ex. 1006 at 15–17]). Following the loading and wash steps, a different solution, typically one of low pH, is applied onto the column to elute the antibody"). Linhult also teaches a cleaning in place step using an alkaline cleaning agent. "The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min, resulting in a total exposure time of 7.5 h for Z(F30A) and mutants thereof." Ex. 1004, 4.

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Abrahmsén teaches using an IgG bound column for purifying the dimeric Z fragment from a supernatant. Ex. 1005, 9:60–10:15; Ex. 1002

¶¶ 64–68. Abrahmsén affinity purification protocol is as follows:

The supernatant was passed through the column at a speed of 12 ml/h and the amount of IgG binding material [i.e. the Z fragment] was analyzed before and after it was run through the column. The bound material was washed with TS [(150 mM NaCl 50 mM tris HC pH 7.5)] supplemented with 0.05% Triton X-100 and then TS and finally with 0.05 M ammonium acetate before elution with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.

Ex. 1005, 10:8–16. The contacting step in Abrahmsén’s protocol occurs when the “supernatant was passed through the column,” the adsorbing step occurs before or during the time “[t]he bound material was washed,” and the “elution” step occurs when the column is treated “with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.” *Id.* Although Abrahmsén column has IgG bound to the column, instead of an SPA domain, the reference still teaches the common chromatography steps of (1) contacting, (2) adsorbing, and (3) eluting a target molecule.

Hober teaches that “protein monomers can be combined-into multimeric proteins, such as dimers, trimers, tetramers, pentamers etc.”

Ex. 1006, 11. These monomer units can be linked with stretches of amino acids ranging from 0–15 amino acids. *Id.* Hober teaches

a matrix for affinity separation, which matrix comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which protein at least one asparagine residue has been mutated to an amino acid other than glutamine. . . The mutated protein ligand is preferably an Fc fragment-binding protein, and can be used for selective binding of IgG. . . the ligands present on the solid support comprising a multimer.

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Ex. 1006, 13. Hober describes a typical chromatographic run cycle consisting of: sample application of 10 mg polyclonal human IgG; extensive washing-out of unbound proteins; elution at 1,0 ml/min with elution buffer; followed by Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0,5 M NaOH of 1 hour. *Id.* at 37.

Patent Owner does not dispute that the references disclose the recited chromatography process of contacting, adsorbing, and elution as we have just described here. *See generally* PO Resp., *see id.* at 7 (citing Ex. 1002 ¶ 24; Ex. 2025 ¶ 43).

(b) Chromatography Matrix

The dispute between the parties is whether a person of ordinary skill in the art would have modified the process disclosed in Linhult using the G29A modified SPA C domain as disclosed in Abrahmsén. Pet. 22 (“Abrahmsén unequivocally discloses performing a G29A mutation on SPA’s C domain.”); *See* PO Resp. 17–54.

Linhult discloses a process for isolating one or more target compound(s) using chromatography matrices (solid support) comprising SPA ligands. Pet. 16–32. Linhult explains that SPA is a cell surface protein expressed by *Staphylococcus aureus* and consists of five highly homologous domains (E, D, A, B, and C). Ex. 1004, 1. Each of “[t]he five SPA domains show[s] individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” *Id.* (bracketing in original). “Due to the high affinity and selectivity of SPA, it has a widespread use as an affinity ligand for capture and purification of antibodies.” Ex. 1004, Abst., *see also*

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id. at 1 (“SPA has a widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.”).

Linhult explains that, in column chromatography, sodium hydroxide (NaOH) is probably the most extensively used cleaning agent for removing contaminants such as nucleic acids, lipids, proteins, and microbes, and a CIP step is often integrated in the protein purification protocols using chromatography columns. Ex. 1004, 1. “Unfortunately, protein-based affinity media show high fragility in this extremely harsh environment, making them less attractive in industrial-scale protein purification. SPA, however, is considered relatively stable in alkaline conditions.” *Id.* at 2. Linhult explains that the combination of asparagine with a succeeding glycine is the most sensitive amino acid sequence to alkaline conditions. *Id.* Linhult teaches that “[a]n exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is [sensitive to alkaline conditions and is also] a cleavage site for hydroxylamine.” *Id.*

Petitioner’s expert, Dr. Cramer avers that the “Z” domain referenced in Linhult refers to a synthetic version of the wild-type (i.e., natural) B domain of SPA, in which the naturally occurring glycine in the Asn₂₈-Gly₂₉ dipeptide sequence is replaced by an alanine residue to create an Asn₂₈-Ala₂₉ dipeptide sequence. Ex. 1002 ¶ 30 (citing Ex. 1004, 2, Fig. 1(a); Ex. 1007, 3, Fig. 1); ¶ 31 (citing Ex. 1005). We credit Petitioner’s expert, Dr. Cramer for establishing that the C domain sequence disclosed in Linhult contains 55 amino acids in SEQ ID NO: 1 as claimed. Ex. 1002 ¶ 106 (showing a sequence alignment), *see also id.* ¶ 292 (showing sequence alignment of domain C of Abrahmsén with SEQ ID NO: 1).

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According to Abrahmsén, the IgG binding domains of E, D, A, B, and C domains of SPA were known. *See* Ex. 1005, 3:25–35, 4:34–37, Fig. 2. Relying on “computer analysis [Abrahmsén] surprisingly showed that the Gly in the Asn-Gly dipeptide sequence could be changed to an Ala. This change was not obvious as glycines are among the most conserved amino acids between homologous protein sequences due to their special features.” *Id.* at 5:7–9. Abrahmsén teaches that, in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. Thus, Abrahmsén provides motivation for making this mutation in any of the IgG binding domains of E, D, A, B, and C domains of Staphylococcal protein A. Abrahmsén teaches recombinant DNA fragments coding for any of the E, D, A, B, and C domains of Staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon. *Id.* at 2:33–37.

Abrahmsén, like Linhult, exemplifies the cloning of and expression of the Z-fragment. Ex. 1005, 7:65–10:56. Abrahmsén teaches that “the Z-region is the part of the Z-fragment coding for the IgG binding domain.” *Id.* at 3:39–41. Abrahmsén purifies the recombinant Z protein using an IgG column. *Id.* at 10:26–28. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén, therefore, reasonably suggests making multimeric constructs. Abrahmsén also uses column chromatography to purify a Z domain containing protein.

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Hober teaches a multimer ligand that

also comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A. In this embodiment, it is preferred that asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids. In an embodiment advantageous for structural stability reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to, an alanine residue. Also, it is advantageous for the structural stability to avoid mutation of the asparagine residue in position 52, since it has been found to contribute to the α -helical secondary structure content of the protein A molecule.

Ex. 1006, 12, *see also id.* at 9 (“SEQ ID NO 1 defines the amino acid sequence of the B-domain of SpA”).

Here, the teachings of Linhult, Abrahmsén, and Hober suggest mutating the glycine at position 29 for an alanine in any one of the IgG binding domains of E, D, A, B, or C of SPA in order to avoid protein degradation. Ex. 1004, 2; Ex. 1005, 5:4–9. We, therefore, agree with Petitioner that the art expressly suggests that the glycine codon can be mutated for an alanine codon in any one of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111). Attaching any one of SPA mutated IgG binding domains E D A B or C to a matrix using “known ligand-construction methods to yield a predictable result[] (e.g., the claimed affinity chromatography matrix)” would have been obvious. Pet. 25 (citing Ex. 1002 ¶ 130). As the Federal Circuit has explained, “[w]here a skilled artisan merely pursues ‘known options’ from ‘a finite number of identified, predictable solutions,’ the resulting invention is obvious under Section 103.” *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421).

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Accordingly, we agree with Petitioner that the combination of Linhult Abrahmsén, and Hober expressly suggests mutating the glycine codon for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111). We also agree that the cited art teaches using these mutant SPA domains in column chromatography for the isolation of antibodies.

We address Patent Owner’s contentions below.

(2) *Response*

(a) *Matrix*

We do not find Patent Owner’s argument that the Petition fails to identify a reason to select domain C persuasive. PO Resp. 17–38. Specifically, we are not persuaded by Patent Owner’s contention that just because nobody was working on domain C at the time the invention was filed, one of skill in the art would not have been motivated to select domain C. *See* PO Resp. 31 (“A general recognition that there exist five naturally occurring protein A domains is not a motivation to use each of them as a starting point for the claimed mutations”).

Petitioner’s articulated obviousness ground is premised on the knowledge that *any one* of the five SPA IgG binding domains are known to bind IgG and can function as a ligand for the purification of antibodies. Linhult and Abrahmsén both expressly suggest that the glycine codon at position 29 can be mutated for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Ex. 1004, 2; Ex. 1005, 2:32–37. Here, the SPA IgG binding domains comprise a short list of 5 members: E, D, A, B, or C. Of these 5 members, the glycine at position 29 in domain B has already been mutated to an alanine to create a domain Z which has been shown to

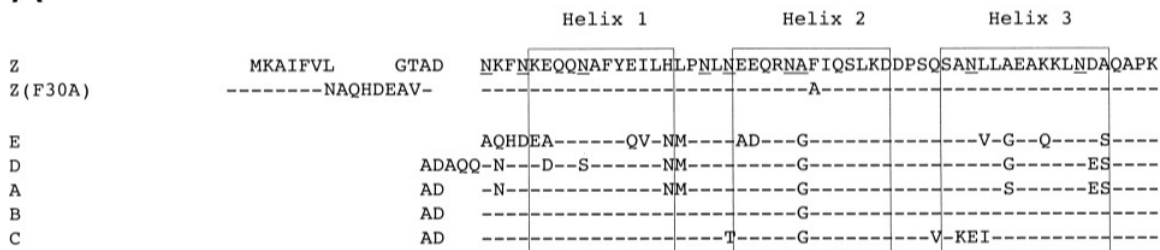
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retain IgG binding activity. Ex. 1004, 6 (Fig. 3). Figure 1A of Linhult is reproduced below.

A



Linhult's Figure 1A, reproduced above, shows the amino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C). The three boxes show the α -helices. Ex. 1004, 2; Ex. 1005, Fig. 2.

Here, Linhult and Abrahmsén show that the IgG binding domains of SPA – E, D, A, B, and C share many structural similarities. *See* Ex. 1004, 2 (Fig. 1(a) (reproduced above)); Ex. 1005, 3:25–35. As discussed in our Institution Decision (Dec. 30–31), there are a finite number—five (5)—SPA IgG binding domains and each possesses the dipeptide sequence Asp-Gly known to be a target for alkaline protein degradation. Therefore, the solution of mutating the glycine at position 29 for an alanine to remove the alkaline sensitive sequence is not a product of innovation, but of ordinary skill and common sense. *See Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421). It is well established that

[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate

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making them to try to obtain compounds with improved properties.

In re Deuel, 51 F.3d 1552, 1558 (Fed. Cir. 1995).

There is also an express teaching in both Linhult and Abrahmsén to mutate the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which further supports why one of skill in the art would have reason to incorporate the mutation into any one of the IgG binding domains that has the Asn-Gly dipeptide. *See, e.g., SIBIA Neurosciences, Inc. v. Cadus Pharm. Corp.*, 225 F.3d 1349, 1358–59 (Fed. Cir. 2000) (stating that an express teaching in the prior art suggesting a particular modification establishes obviousness).

Because the G29A modification would have provided ligands that are less susceptible to alkaline conditions and are resistant to hydroxylamine cleavage, Petitioner has provided a sufficient evidence-backed reason for making the modification in any one of the domains. Pet. 16–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

(b) Reasonable Expectation of Success

We are not persuaded by Patent Owner’s contention that there is no reasonable expectation of success in using a G29A mutation in domain C. PO Resp. 48–54.

Linhult explains that removing the Asp–Gly amino acid combination not only results in the removal of the hydroxylamine cleavage site, but also creates a product that is more alkaline resistant. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for

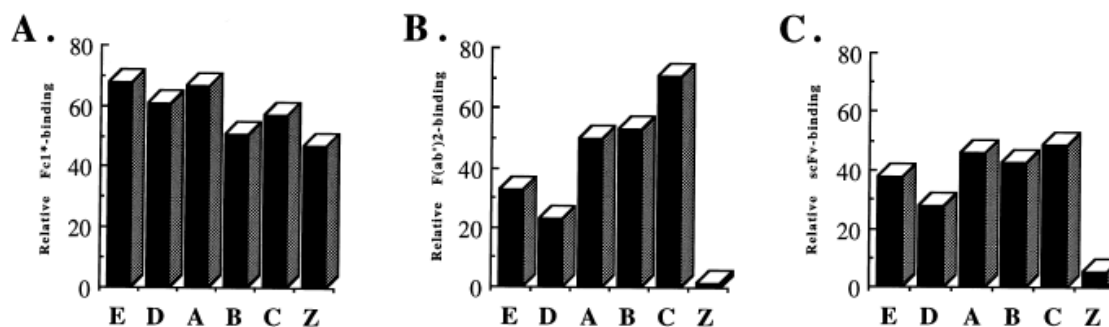
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hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”).

Abrahmsén teaches that this Asn-Gly amino acid combination is present in all five SPA IgG binding domains and that mutating the dipeptide would not interfere with IgG binding. Ex. 1005, 4:56–58 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine. [T]his sequence is kept intact in all five IgG binding domains of protein A. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”). Abrahmsén’s conclusion that the mutation would not interfere with binding to IgG is supported by Abrahmsén (*see* Ex. 1005, 9:60–10:35), Linhult (*see* Ex. 1004, 6 (Fig. 3)), and Jansson (Ex. 2029).⁷ Jansson’s Fig. 3, reproduced below:



Jansson Figure 3 (Panel A), reproduced above, shows a side-by-side comparison of Fc1*, Fab, and scFv binding to SPA domains. The figure shows that the single G29A mutation between domain B and domain Z

⁷ Patent Owner cites Jansson (Ex. 2029) for the position that domain Z has negligible binding to Fab. *See* PO Resp. 40–41. However, claims 1 and 14 are not limited to Fab binding. Indeed, the claims do not even require IgG binding.

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results in a protein that is able to bind IgG.⁸ Comparing panel A - domain B domain with panel A - domain Z, the relative binding remains close to 50%, indicating that the G29A mutation between domains B and C does not interfere with IgG binding. Ex. 2029, 6. This result is predicted by Abrahmsén's computer modeling and substantiated by Abrahmsén domain Z purification and Linhult's IgG purification. *See* Ex. 1005, 4:56–58, 9:60–10:35; Ex. 1004, 6 (Fig. 3).

“Obviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success.” *In re Droge*, 695 F.3d 1334, 1338 (Fed. Cir. 2012) (quoting *In re Kubin*, 561 F.3d 1351, 1360 (Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894, 903–04 (Fed.Cir.1988)); *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016) (explaining that the expectation of success issue involves a showing of “a reasonable expectation of achieving *what is claimed*”) (emphasis added). “Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention.” *Pharma Stem Therapeutic, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1363–1364 (2007).

Here, the record supports that each individual SPA domain, including the C domain, has affinity for IgG antibodies. Ex. 1004, 1 (“The five SPA

⁸ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the “IgG control” in Jansson. Patent Owner's counsel explains that “Part A is Fc binding. So that is, I believe the way they did this experiment was with Fc fragments, but it's generally acknowledged, you know, these antibodies all have an Fc domain if they're a whole antibody and that reflects the fact that all of these domains A, B, C, D and E and domain Z, which is B with the G29A mutation, retain this Fc binding.” Tr. 70:6–11.

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domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” (bracketing in original)). Abrahmsén suggests making a mutation of Asn-Gly coding constellation in *any one* of the SPA domains by replacing a glycine codon with an alanine codon to remove the Asn-Gly dipeptide sequence known to be sensitive to hydroxylamine degradation. *See* Ex. 1005, 4:56–5:16, *see also id.* Fig. 2 (showing the Asn-Gly coding constellation in all SPA domains); Ex. 1006, 2 (“the shortest deamidation half times have been associated with the sequences –asparagine–glycine and – asparagine–serine”). Abrahmsén’s confirms that a G29A mutation on SPA would not interfere with folding of SPA protein and binding to antibodies. Ex. 1005, 5:13–16 (“by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”), 9:60–10:35 (using IgG columns to purify protein Z dimers). Abrahmsén’s computer modeling suggests that IgG binding is not impacted by the mutation, and this is confirmed by Linhult’s experiments showing that the G29A mutant of domain B (a.k.a. domain Z) binds IgG. Ex. 1004, 6 (Fig. 3); *see also* Ex. 1005, 9:60–10:35.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

We are not persuaded by Patent Owner’s contention that the information gained by computer modeling of the SPA native domain B-IgG

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crystal structure could not be extrapolated to other SPA domains that are structurally very similar.

As Petitioner's expert, Dr. Cramer, explains

[i]t was well known that the researchers who developed the Z domain based on the wild-type B domain (rather than any of the other four SPA domains) did so for two reasons. (*See, e.g.*, Ex. 1007 at 109.) First, a crystal structure of the wild-type B domain binding to an antibody happened to be available in 1981 for analysis. (*See, e.g., id.*; Ex. 1005 at col. 4:56-68; Ex. 1017.) And, second, *their work would be informative of mutations that could be done on all five of the highly homologous SPA domains more generally.* (*See, e.g.*, Ex. 1005 at col. 2:32-37; Ex. 1007 at 109; Ex. 1008 at 639, Fig. 1.)

Ex. 1002 ¶ 33 (emphasis added). Dr. Cramer further explains that “[t]hey did the computer modeling based on that complex because that’s the crystal structure that they had. It wasn’t done because the B domain is special. . . . And then there’s several other places where they state clearly that they could also do the other domains with expected similar results.” Ex. 2015, 138:8–22.

We credit Dr. Cramer’s testimony here and agree with Petitioner that, when taken together, the teachings of Linhult, Abrahmsén, and Hober provide a reasonable expectation of success at arriving at a chromatography composition that contains the SPA domain C ligand with the G29A mutation that can be used in a process for purifying IgG. Pet. 24 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 124–126), *see id.* at 48–49 (citing Ex. 1006, 10–12; Ex. 1002 236–263).

(c) No Teaching Away

We are also not persuaded by Patent Owner’s contention that the art teaches away from the G29A substitution because it interferes with Fab

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binding. *See* PO Resp. 41 (citing Ex. 2009 at 2; Ex. 2010 at 2; Ex. 2012 at 25–26; Ex. 2029 at 7); Sur-reply 9.

Neither claim 1 nor claim 14 recite a need to bind the Fab region of an antibody or that the target molecule is Fab. All that is required by these claims is that they adsorb a target molecule and that you can elute the target molecule from the matrix. The target molecule, therefore, can reasonably encompass IgG.

Petitioner’s articulated rationale is that there was an expectation that the composition binds antibodies, including monoclonal antibodies, and therefore, would be useful in a process of for isolating antibodies. Petitioner contends that:

A POSA would have also reasonably expected such a combination to achieve a process for isolating one or more target compounds using the recited affinity chromatography matrix given the well-known fact that each individual SPA domain, including the C domain, has affinity for antibodies (Ex. 1004, [1]) as well as *Abrahmsén*’s confirmation that a G29A mutation on SPA “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1005, 5:13-16). (Ex. 1002 ¶131.)

Pet. 25; Reply 8 (“A POSA would not have been motivated only by Fab-binding ability, as even Dr. Bracewell agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’ (Ex. 1057, 75:17-76:4, 113:23-114:11, 157:24-158:9; Ex. 1061 ¶29)”).

The law does not require that the teachings of the reference be combined for the reason or advantage contemplated by the inventor, as long as some suggestion to combine the elements is provided by the prior art as a whole. *In re Beattie*, 974 F.2d 1309, 1312 (Fed. Cir. 1992); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976); *see In re Kemps*, 97 F.3d 1427, 1430

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(Fed. Cir. 1996) (“[T]he motivation in the prior art to combine the references does not have to be identical to that of the applicant to establish obviousness.”).

Here, Linhult teaches that “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” Ex. 1004, 1 (bracketing in original) (citation omitted). Linhult, therefore, teaches that *any one* of the SPA IgG binding domains E, D, A, B, or C can bind the Fc region of an antibody and can therefore be used as a ligand for purifying IgG antibodies. In addition, the combination of Linhult and Abrahmsén suggests making the G29A mutation in each of the domains because it would provide ligands that are less susceptible to protein degradation. Ex. 1004, 2; *see also* Ex. 1005, 2:33-37 (“[A] recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.”).

Patent Owner contends that the G29A mutation would lead to a reduction in the Fab binding of domain C, and therefore, would lead away from making the mutation. PO Resp. 40–41 (citing Ex. 2010, 2; Ex. 2011, 25; Ex. 2012, 25–26; Ex. 2013, 2–3; Ex. 2025 ¶¶ 105–109; Ex. 2029, 6–7). Patent Owner’s cited references are directed to Fab binding. But claims 1 and 14 are not limited to Fab binding. Showing that the G29A mutation interferes with Fab binding does not discredit the ability of a mutated SPA domains E, D, A, B, or C to bind the Fc portion of IgG. *See, e.g.*, Ex. 2013, 3 (“The site responsible for Fab binding is structurally separate from the

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domain surface that mediates Fcγ⁹ binding.”). Accordingly, we are not persuaded by Patent Owner’s contention that with respect to claims 1 and 14 that the art teaches away from Petitioner’s proposed combination.

(d) Additional Modifications

We also disagree with Patent Owner’s contention that the ordinary artisan would not stop with a single G29A mutation in a SPA domain. *See* PO Resp. 44–48. Here, Abrahmsén expressly suggests making only a single mutation. Specifically, Abrahmsén contemplates “a recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon” without additional mutations. Ex. 1005, 2:33–37.

(3) Summary

We find that Petitioner has shown by a preponderance of the evidence that the combined teachings of at least Linhult and Abrahmsén suggests the use of *any one* of the SPA IgG binding domains E, D, A, B, or C as the starting ligand for purifying IgG antibodies, and that making the G29A mutation in *any one* of the domains would have been obvious because it would have provided ligands that are less susceptible to alkaline conditions

⁹ Fcγ is the constant region of IgG involved in effector function. Ex. 2013, 1. Specifically, “[t]he Fcγ binding site has been localized to the elbow region at the CH2 and CH3 interface of most IgG subclasses, and this binding property has been extensively used for the labeling and purification of antibodies.” *Id.* In other words, Fcγ and Fc terminology are used interchangeably in the art.

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and are resistant to hydroxylamine cleavage. Pet. 16–25; Reply 3–5;

Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

Having considered the evidence and argument of record, which we have described above and find persuasive, we determine that Petitioner has shown by a preponderance of evidence of record that the combination of Linhult, Abrahmsén, and Hober teaches each of the limitations of claims 1 and 14. Petitioner not only has articulated a sufficient motivation for making the combination but has also established that there is a reasonable expectation of success for the binding of an IgG antibody to a SPA domain that contains an G29A mutation.

b) Claims 4 and 17

Petitioner argues that “[t]he ‘capab[ility] of binding to the Fab part of an antibody,’ as recited in claims 4 and 17, is an inherent property of the claimed C(G29A)-based SPA ligand.” Pet. 28 (citing Ex. 1002 ¶¶ 141–148). Petitioner contends that a person of ordinary skill in the art did not need to recognize the Fab binding property of domain C to be motivated to select that domain for modification. “A POSA would not have been motivated only by Fab-binding ability,[] as even Dr. Bracewell [Patent Owner’s expert] agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’” Reply 8 (citing Ex. 1057, 75:17–76:4, 113:23–114:11, 157:24–158:9; Ex. 1061 ¶ 29).

Patent Owner argues that

[t]he very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding “negligible” when implemented in Domain B. Ex. 2009 at 2; *see also, e.g.*, Ex. 2010 at 2 (“Fab binding activity is located to a region determined by

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helices 2-3, including the position mutated to yield the Z domain.”); Ex. 2011 at 25 (“[I]t only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine to an alanine”); Ex. 2012 at 25-26 (“[D]omain Z containing a single G29A-substitution compared to domain B exhibits little or no [Fab] binding. This might be due to the substitution since the C_β of the alanine would perturb the interaction between the two molecules.”).

PO Resp. 41.

Because claim 4¹⁰ is directed to a “[a] process for isolating one or more target compound(s)” and identifies that the target compound is “the Fab part of an antibody” (Ex. 1001, 15:63–64) the prior art needs show a reasonable expectation that a mutated SPA ligand binds Fab. Without such a showing, there is no reasonable expectation that the process would result in the purification of a Fab target. In other words, because the claims are process claims, Petitioner needs to establish that a mutated SPA domain would reasonably bind a Fab fragment.

We agree with Petitioner, that Linhult establishes that a G29A mutation in domain B (resulting in domain Z) does not interfere with IgG binding. *See* Ex. 1004, 6 (Fig. 3 showing IgG binding with domain Z). Linhult, however, is silent with respect to domain Z’s ability to bind to Fab fragments. *See generally* Ex. 1004. Abrahmsén similarly establishes domain Z binding to IgG but is also silent with respect to domain Z binding Fab. *See generally* Ex. 1005. Hober also does not disclose Fab binding of a mutant SPA domain. *See generally* Ex. 1006. Thus, each of Linhult,

¹⁰ Claim 14 and 17 recite similar limitations.

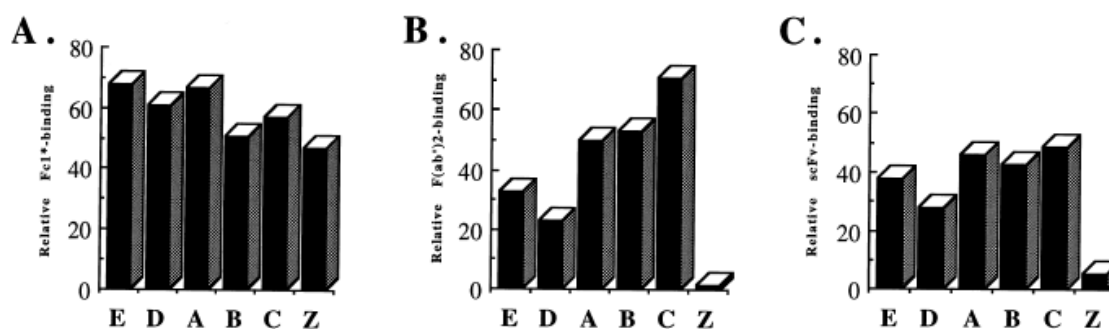
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Abrahmsén, or Hober is silent with respect to Fab binding to a mutated SPA domain.

Jansson (Ex. 2029), cited by Patent Owner, supports the position that Fab binding to mutated SPA domains is unpredictable. Jansson, just like Linhult, recognizes that “[a]ll [SPA] domains bound to a recombinant human IgG1 Fc fragment with similar strength. For the first time, binding to human Fab was demonstrated for all *native SPA domains*, using both polyclonal F(ab')₂ and a recombinant scFv fragment as reagents.” Ex. 2029, Abstract (emphasis added). Jansson, however, establishes that “the engineered Z domain showed a considerably lower affinity for Fab as compared to the native domains.” *Id.* Jansson Fig. 3, reproduced below, shows that the G29A mutation results in a loss of Fab binding ability.



Jansson Figure 3, reproduced above, shows the side-by-side comparison of Fc1*¹¹, Fab, and scFv binding and confirms what was already suggested in Linhult, Abrahmsén, and Hober – that a composition containing the G29A mutation in a SPA domain can bind IgG. Ex. 2029, 6 (Fig. 3 (*compare* Panel A- domain B, *with* Panel A- domain Z)). Panel B in Jansson Figure 3, however, shows that the single G29A mutation between domain B and

¹¹ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the IgG control in Jansson. Fc1* is functionally equivalent to Fcγ in SPA binding.

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domain Z results in the loss of Fab binding. Ex. 2029, 6 (Fig. 3 (*compare* Panel B- lane B, *with* Panel B- lane Z)). At the time the invention was made it was also known that “[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding.” Ex. 2013, 3. Thus, on this record, establishing that a mutation that does not interfere with IgG binding does not inform one of skill in the art about the ability of a mutated SPA domain to bind Fab.

We, therefore, agree with Patent Owner’s contention that based on the prior art, the Fab binding capacity was unknown with the modification as suggest by the combination of Linhult, Abrahmsén, and Hober.¹² Patent Owner has provided evidence that Fab binding capacity of a mutated SPA domain protein is unpredictable. *See* PO Resp. 56–57 (Ex. 2029, 5–6). The disclosures in the prior art, therefore, support Patent Owner’s position that “the SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” PO Resp. 54–55 (citing Ex. 2025 ¶ 123; Ex. 2030, 18–19).

Because the art does not support the conclusion that G29A mutation in a SPA domain ligand binds Fab, Petitioner has not established by a

¹² In *JSR Corporation et al. v. Cytiva Bioprocess R&D AB et al.*, IPR2022-00036, Paper 41 at 44–49 (PTAB April 19) (Final Written Decision) we determined that the capability of SEQ ID NO:1 to bind Fab is an inherent feature of the structure claimed. The present claims, however, are directed to a method of isolating Fab which requires prior knowledge that the ligand binds Fab. Fab is a digestion product of a whole IgG molecule treated with papain and is not naturally found in IgG samples. *See above* I.F. In other words, when isolating IgG with a column containing SEQ ID NO: 1, there would be no elution of Fab because the fragments are not present in an IgG containing sample.

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preponderance of the evidence of record that the process of isolating Fab target using a mutated SPA domain C ligand would have been obvious based on the combined teachings of Linhult, Abrahmsén, and Hober.

c) Claims 3, 16, and 28–30

Claims 3, 27, and 28 depend either directly or indirectly from claim 1, and claims 16, 29, and 30 depend either directly or indirectly from claim 14. Claim 3 and 16 recite the additional limitation “wherein the chromatography matrix has retained at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.” Ex. 1001, 15:52–62. Claim 27 and 29 recite “comprising a step of exposing the chromatography matrix to 0.1 to 0.5 M NaOH.” Claim 29 and 30 recite “repeated exposure of the chromatography matrix to the NaOH for at least 80 cycles.”

We are not persuaded by Patent Owner’s argument that “none of Petitioners’ cited references actually describe a C(G29A)-based SPA ligand, let alone provide alkaline stability data or test results concerning the same, the POSA is simply left to guess at how such a ligand would perform.” PO Resp. 50 (citing Ex. 2025 ¶¶ 121–124).

For the reasons discussed above (II.E.5.2.a), we find that Petitioner has shown by a preponderance of evidence that there is a reason to make the G29A mutation in any one of the SPA IgG binding domains, including domain C, and that the use of the mutated protein would reasonably result in a matrix that can be used to purify IgG. Linhult teaches a CIP protocol with at 0.1 to 0.5M NaOH was a well-known and conventional technique. Pet. 30 (citing Ex. 1004, 1–2, 4–5; Ex. 1002 ¶ 152); Ex. 1004, 6 (“Figure 3, the Z(N23T) mutant shows higher resistance to alkaline conditions than the Z domain when exposed to high pH values.”); *see also* Ex. 1006, 39

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(“Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0,5 M NaOH of 1 hour”). “Between each cycle [in Linhult], a CIP-step was integrated. The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min.” Ex. 1004, 4. “After 16 [CIP] cycles, giving a total exposure time of 7.5 h, the column with the Z(F30A)-matrix shows a 70% decrease in capacity.” Ex. 1004, 5; *see also* Ex. 1006, 39 (“Each cycle [in Hober] was repeated 21 times resulting in a total exposure time between the matrix and the sodium hydroxide of 20 hours for each different matrix”). Both Linhult and Hober recognize that repeated exposure of a SPA chromatography ligand leads to a reduction of the binding capacity over time. That Linhult recognizes that additional mutations could further improve alkaline stability does not detract from Linhult’s teaching that a composition containing the single G29A mutation in SPA domain B retains IgG binding. Ex. 1004, 6, *see id.* at 4 (“The Z-domain already possesses a significant tolerance to alkaline conditions.”).

Petitioner has shown by a preponderance of the evidence of record that there is a reason for making the G29A mutation in *any one* of the four remaining SPA domains in order to produce a SPA product that is more alkaline stable and would reasonably bind IgG. *See* Pet. 26–28, 30; Reply 20–21; *see* Ex. 1002 ¶¶ 115–119.

d) Claims 2, 12, 15, and 25

Claims 2 and 12 depend from claim 1, and claims 15 and 25 depend from claim 14. Claim 2 and 15 recite the additional limitation that “ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.” Claims 12 and 25 recite the additional element that “the ligand comprises an amino acid sequence that comprises 2-8 of the polypeptides.”

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Petitioner asserts that Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims. *See* Pet. 25–28, 30.

Patent Owner does not offer arguments addressing Petitioner’s substantive showing with respect to the additional elements added by dependent claims 2, 12, 15, and 25. *See generally* PO Resp.

We have reviewed Petitioner’s arguments and the underlying evidence cited in support and determine that Petitioner establishes that of Linhult, Abrahmsén, and Hober teaches the additional limitations of these dependent claims. Pet. 26 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 112–114), *see id.* at 30 (citing Ex. 1002 ¶¶ 149–150); Ex. 1004, 4 (“a multimerization of the domain to achieve a protein A–like molecule”); Ex. 1005, 9:15–10:35. Hober also teaches that monomeric mutant proteins can be combined into multimeric proteins, such as dimers, trimers, tetramers, pentamers, and other multimers. Ex. 1006, 11. Hober also discloses that the multimer comprises mutant monomer units “linked by a stretch of amino acids preferably ranging from 0 to 15 amino acids, such as 5-10.” *Id.*

The preponderance of evidence of record supports Petitioner’s contentions with respect to claims 2, 12, 15, and 25.

6. Summary

For the foregoing reasons, we determine that Petitioner has shown by a preponderance of evidence that of claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the ’142 patent are unpatentable based on the combination of Linhult, Abrahmsén, and Hober.

For the reasons discussed above, Petitioner has not shown by a preponderance of evidence that of claims 4 and 17 of the ’142 patent are unpatentable.

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F. *Other Asserted Grounds*a) *The '041 IPR Grounds Based on Other Various Combinations of Linhult, Abrahmsén, and Hober*

Petitioner also asserts that claims 1–4, 12, 14–17, and 25 are unpatentable as obvious over Linhult and Abrahmsén (Pet. 18–30); that claims 1–7, 10–20, and 23–26 are unpatentable as obvious over Linhult and Hober (*id.* at 30–48); and that claims 1–7, 10–20, 23–26 are unpatentable as obvious over Abrahmsén and Hober (*id.* at 49–60) under 35 U.S.C. §103(a).

Because Petitioner has already shown that the challenged claims 1–3, 5–7, 10–16, 18–20, and 23–30 are unpatentable over Linhult, Abrahmsén, and Hober as obvious, as discussed *supra*, we do not reach these claims in these additional asserted grounds as to those claims. *See Beloit Corp. v. Valmet Oy*, 742 F.2d 1421, 1423 (Fed. Cir. 1984) (“The Commission . . . is at perfect liberty to reach a ‘no violation’ determination on a single dispositive issue.”); *Boston Sci. Scimed, Inc. v. Cook Grp., Inc.*, 809 F. App’x 984, 990 (Fed. Cir. 2020) (recognizing that “[t]he Board has the discretion to decline to decide additional instituted grounds once the petitioner has prevailed on all its challenged claims”).

Petitioner has not shown that the challenged claims 4 and 17 are unpatentable over Linhult, Abrahmsén, and Hober. We note that each of Linhult, Abrahmsén, and Hober is silent with respect to Fab binding to a mutant SPA domain. *See above* II.E.5.b. Patent Owner asserts, and we agree, that there is no reasonable expectation that a G29A SPA domain mutant would bind Fab. Specifically, Patent Owner’s cited references show that a G29A mutation in domain B results in a domain Z matrix composition that *does not* bind Fab. *See* Ex. 2029, 6 (Fig. 3 (*compare* Panel B- domain B,

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with Panel B- domain Z)); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”). For the reason discussed above (II.E.5.b), Petitioner has not shown by a preponderance of the evidence of record that a G29A SPA domain mutant would bind a Fab fragment so that a process of using the G29A SPA domain ligand would reasonably result in the purification of the Fab target. Petitioner’s deficiency with respect to claims 4 and 17 persists whether the grounds are based on the combination of Linhult, Abrahmsen, and Hober or the related combinations Linhult and Abrahmsen; Linhult and Hober; or Abrahmsen combined with Hober.

b) The '044 IPR Grounds Based on Various Combinations of Berg¹³, Linhult, Abrahmsén, and Hober

Petitioner asserts that claims 1–7, 10–20, and 23–30 are unpatentable as obvious over Berg¹⁴ and Linhult ('044 IPR Pet. 18–30); that claims 2, 3, 15, and 16 are unpatentable as obvious over Berg, Linhult, and Hober (*id.* at 30–48); that claims 1, 2, 5–7, 10–15, 18–20, and 23–26 are unpatentable as obvious over Berg and Abrahmsén (*id.* at 49–60); and that claims 2–4, 15–17, and 27–30 are unpatentable as obvious over Berg, Abrahmsén, and Hober (*id.* at 49–60) under 35 U.S.C. §103(a).

¹³ Berg et al., US 2006/0134805 A1, published June 22, 2006. Ex. 1018.

¹⁴ We recognize that there is a dispute between the parties whether Berg qualifies as a 35 U.S.C. §102(a) date reference or a §102(b) date reference. *See* Pet; PO Resp., Reply, and Sur-reply. Because we do not need to reach these additional asserted grounds based on Berg beyond addressing whether Berg teaches Fab binding in the context of a mutant SPA domain ligand to establish that Berg does not address the deficiency of the Linhult, Abrahmsén, and Hober combination, we do not need to address the prior art status of Berg.

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Berg relates to a chromatography matrix to which antibody-binding protein ligands are immobilized. Ex. 1018, Abstract. Petitioner relies on single paragraph in Berg, paragraph 29, for teaching antibody binding ligands including SPA domain C. *See* '044 IPR Pet. 23, 45. The remainder of the Berg reference is directed to the structure of the chromatography matrix. *See generally* Ex. 1018. A review of Berg shows that SPA is mentioned at three locations in the reference. *See* Ex. 1018 ¶¶ 28, 29, and claim 12. Paragraph 29 of Berg suggests using ligands made up of one or more domains A, B, C, D, and E, and preferably domain B and/or domain C. Ex. 1018 ¶ 29. Just like Linhult, Abrahmsén, and Hober, Berg also does not say anything about the ability of a mutant SPA domain ligand, including a domain C ligand, to bind Fab.

Patent Owner, however, cites prior art references to establish that at the time the invention was made there was no expectation that a G29A SPA domain mutant would bind Fab. *See above* II.E.5.b; *see* '044 IPR PO Resp. 45, 55, 56, 60. Specifically, Patent Owner's cited references showing that a G29A mutation in domain B results in a domain Z matrix composition that does not bind Fab. *See id.*; Ex. 2029, 6 (Fig. 3 (compare Panel B- domain B, with Panel B- domain Z)); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”).

Berg, therefore, does not address Fab binding in the context of a mutant SPA domain ligand, specifically domain C, nor does Berg explain why one of ordinary skill in the art would have reasonably expected domain C to retain the ability to bind Fab when other SPA domain mutants do not retain this feature. Because Berg does not address the deficiency of

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Linhult, Abrahmsén, and Hober as identified by Patent Owner and discussed above (II.E.5.b), any combination of Berg in conjunction with Linhult, Abrahmsén, and/or Hober would not address the missing limitation of claims 4 and 17. Therefore, we do not reach these additional asserted grounds based on Berg beyond addressing whether Berg teaches this missing limitation. *See Beloit Corp.*, 742 F.2d at 1423.

III. CONCLUSION¹⁵

For the foregoing reasons, we determine that Petitioner has demonstrated by a preponderance of the evidence that claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the '142 patent are unpatentable, and that claims 4 and 17 have not been shown unpatentable on the bases set forth in the following table.

¹⁵ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this decision, we draw Patent Owner's attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. See 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. See 37 C.F.R. § 42.8(a)(3), (b)(2).

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In summary:

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–4, 12, 14–17, 25	103(a)	Linhult, Abrahmsén ¹⁶		4, 17
1–7, 10–20, 23–26	103(a)	Linhult, Hober ¹⁷		4, 17
1–7, 10–20, 23–30	103(a)	Linhult, Abrahmsén, Hober	1–3, 5–7, 10–16, 18–20, 23–30	4, 17
1–7, 10–20, 23–26	103(a)	Abrahmsén, Hober ¹⁸		4, 17
Overall Outcome			1–3, 5–7, 10–16, 18–20, 23–30	4, 17

¹⁶ As explained above (II.F.a), we do not reach claims 1–3, 12, 14, 15, and 25 in this '041 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

¹⁷ As explained above (II.F.a), we do not reach claims 1–3, 5–7, 10–16, 18–20, 23–26 in this '041 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

¹⁸ As explained above (II.F.a), we do not reach claims 1–3, 5–5, 10–16, 18–20, and 23–26 in this '041 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1-7, 10-20, 23-26	103(a)	Berg, Linhult ¹⁹		4, 17
2, 3, 15, 16	103(a)	Berg, Linhult, Hober ²⁰		
1, 2, 5-7, 10-15, 18-20, 23-26	103(a)	Berg, Abrahmsén ²¹		
2-4, 15- 17, 27- 30	103(a)	Berg, Abrahmsén, Hober ²²		4, 17
Overall Outcome			1-3, 5-7, 10-16, 18-20, 23-30	4, 17

¹⁹ As explained above (II.F.b), we do not reach claims 1-3, 5-7, 10-16, 18-20, and 23-26 in this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²⁰ As explained above (II.F.b), we do not reach this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²¹ As explained above (II.F.b), we do not reach this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²² As explained above (II.F.b), we do not reach claims 2, 3, 15, 16, and 27-30 in this '044 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

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IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the preponderance of the evidence of record has shown that claims 1–3, 5–7, 10–16, 18–20, and 23–30 of the '142 patent are found unpatentable;

ORDERED that the preponderance of the evidence of record has not shown that claims 4 and 17 of the '142 patent are found unpatentable; and

FURTHER ORDERED because this is a final written decision, the parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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Paper: 43
Date: May 18, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

JSR CORPORATION and JSR LIFE SCIENCES, LLC,
Petitioner,

v.

CYTIVA BIOPROCESS R&D AB,
Patent Owner.

IPR2022-00042
IPR2022-00045
Patent 10,875,007 B2

Before ULRIKE W. JENKS, SHERIDAN K. SNEDDEN, and
SUSAN L. C. MITCHELL, *Administrative Patent Judges*.

SNEDDEN, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining Some Challenged Claims Unpatentable
35 U.S.C. § 318

IPR2022-00042
IPR2022-00045
Patent 10,875,007 B2

I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review of claims 1–14, 16–32, and 34–37 (“the challenged claims”) of U.S. Patent No. 10,875,007 B2 (Ex. 1001, “the ’007 patent”). We have jurisdiction under 35 U.S.C. § 6, and enter this Final Written Decision pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons set forth below, we determine that JSR Corporation and JSR Life Sciences, LLC (collectively, “Petitioner”) has shown, by a preponderance of the evidence, that some of the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e).

A. *Consolidated Proceedings*

The two captioned proceedings (IPR2022-00042 and IPR2022-00045 (or “the ’045 IPR”)) involve the ’007 patent and challenge the same set of claims. The asserted grounds and prior art contentions are different in each proceeding. Consolidation is appropriate where, as here, the Board can more efficiently handle the common issues and evidence, and also remain consistent across proceedings. Under 35 U.S.C. § 315(d), the Director may determine the manner in which these pending proceedings may proceed, including “providing for stay, transfer, consolidation, or termination of any such matter or proceeding.” *See also* 37 C.F.R. § 42.4(a) (“The Board institutes the trial on behalf of the Director.”). There is no specific Board rule that governs consolidation of cases. But 37 C.F.R. § 42.5(a) allows the Board to determine a proper course of conduct in a proceeding for any situation not specifically covered by the rules and to enter non-final orders to administer the proceeding. Therefore, on behalf of the Director under § 315(d), and for a more efficient administration of these proceedings, we

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consolidate IPR2022-00042 and IPR2022-00045 for purposes of rendering this Final Written Decision.

B. *Evidence*

Petitioner relies upon information that includes the following:

Ex. 1004, M. Linhult, *et al.*, *Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach*, 55 PROTEINS: STRUCTURE, FUNCTION, AND BIOINF., 407–16 (2004) (“Linhult”).

Ex. 1005, L. Abrahmsén, *et al.*, U.S. Patent No. 5,143,844 (issued Sept. 1, 1992) (“Abrahmsén”).

Ex. 1006, S. Hober, PCT Publication No. WO 03/080655 A1 (published Oct. 2, 2003) (“Hober”).

Ex. 1018, H. Berg., U.S. Patent Application Publication No. 2006/0134805 (published June 22, 2006) (“Berg”).

C. *Procedural History*

Petitioner filed a Petition for an *inter partes* review of the challenged claims under 35 U.S.C. § 311. Paper 1¹ (“Pet.”). Petitioner supported the Petition with the Declaration of Dr. Steven M. Cramer. Ex. 1002. Cytiva Bioprocess R&D AB (“Patent Owner”) filed a Patent Owner Preliminary Response to the Petition. Paper 8.

On May 19, 2022, pursuant to 35 U.S.C. § 314(a), we instituted trial (“Decision” or “Dec.” (Paper 10)) to determine whether any challenged claim of the ’007 patent is unpatentable.

¹ We note that the evidence filed in both proceedings is generally consistent in having the same exhibit number. Therefore, we reference exhibits and paper numbers as they appear in the record of IPR2022-00042, unless otherwise noted.

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In IPR2022-00042, Petitioner asserts the following grounds of unpatentability (Pet. 4):

Claim(s) Challenged	35 U.S.C. §²	Reference(s)/Basis
1–11, 20–29	103(a)	Linhult, Abrahmsén
1–14, 16–32, 34–37	103(a)	Linhult, Hober
1–14, 16–32, 34–37	103(a)	Linhult, Abrahmsén, Hober
1–14, 16–32, 34–37	103(a)	Abrahmsén, Hober

In IPR2022-00045, Petitioner asserts the following grounds of unpatentability ('045 IPR Pet. 4):

Claim(s) Challenged	35 U.S.C. §	Reference(s)/Basis
1–14, 16–18, 20–32, 34–36	103(a)	Berg, Linhult
4–8, 10, 19, 22–26, 28, 37	103(a)	Berg, Linhult, Hober
1–3, 9, 10, 12–14, 16–18, 20, 21, 27, 28, 30–32, 34–36	103(a)	Berg, Abrahmsén
4–8, 10, 11, 19, 22, 26, 28, 29, 37	103(a)	Berg, Abrahmsén, Hober

Patent Owner filed a Patent Owner Response to the Petition. Paper 15 (“PO Resp.”). Patent Owner supported the Response with the Declaration of

² The Leahy-Smith America Invents Act (“AIA”) included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the '007 patent issued from an application claims priority from an application filed before March 16, 2013, we apply the pre-AIA versions of the statutory bases for unpatentability.

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Dr. Daniel Bracewell (Ex. 2025). *See* PO Resp., iv (Exhibit List). Petitioner filed a Reply to the Patent Owner Response. Paper 28 (“Reply”). Petitioner supported the Reply with a Reply Declaration from Dr. Steven M. Cramer. Ex. 1061. Patent Owner filed a Sur-reply to Petitioner’s Reply. Paper 34 (“Sur-reply”).

On February 16, 2023, the parties presented arguments at an oral hearing. Paper 35. The hearing transcript has been entered in the record. Paper 39 (“Tr.”).

For the reasons set forth below, we determine that Petitioner has shown by a preponderance of the evidence that claims 1–10, 12–14, 16–28, 30–32, and 34–37 of the ’007 patent are unpatentable, but find that Petitioner has not shown by a preponderance of the evidence that claims 11 and 29 are unpatentable.

D. *Related Matters*

The ’007 patent is at issue in *Cytiva Bioprocess R&D et al. v. JSR Corp. et al.*, Case No. 21-310-RGA (D. Del.). Pet. 2; Paper 5, 1.

In addition to the ’007 patent, Petitioner filed Petitions for *inter partes* review of related U.S. patents as follows: U.S. Patent No. 10,343,142 B2 (“the ’142 patent”) in IPR2022-00041 and IPR2022-00044; and U.S. Patent No. 10,213,765 B2 (“the ’765 patent”) in IPR2022-00036 and IPR2022-00043. Pet. 2–3; Paper 5, 1–2. The ’007 patent is a continuation of the ’142 patent which is a continuation of the ’765 patent. Ex. 1001, code (60).

E. *Subject matter background*

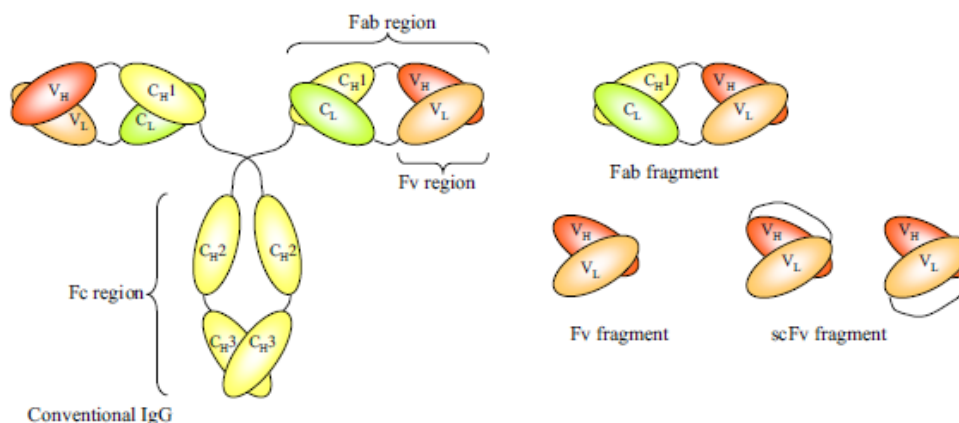
Antibodies (also called immunoglobulins) are glycoproteins, which specifically recognize foreign molecules. These recognized foreign

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molecules are called antigens. Ex. 2001, 1. A schematic representation of the structure of a conventional IgG and fragments is shown below:



The figure (Ex. 2001, 2 (Fig. 1)), reproduced above, shows

the structure of a conventional IgG and fragments that can be generated thereof. The constant heavy-chain domains C_{H1} , C_{H2} and C_{H3} are shown in yellow, the constant light-chain domain (C_L) in green and the variable heavy-chain (V_H) or light-chain (V_L) domains in red and orange, respectively. The antigen binding domains of a conventional antibody are Fabs and Fv fragments. Fab fragments can be generated by papain digestion. Fvs are the smallest fragments with an intact antigen-binding domain. They can be generated by enzymatic approaches or expression of the relevant gene fragments (the recombinant version). In the recombinant single-chain Fv fragment, the variable domains are joined by a peptide linker. Both possible configurations of the variable domains are shown, i.e. the carboxyl terminus of V_H fused to the N-terminus of V_L and vice versa.

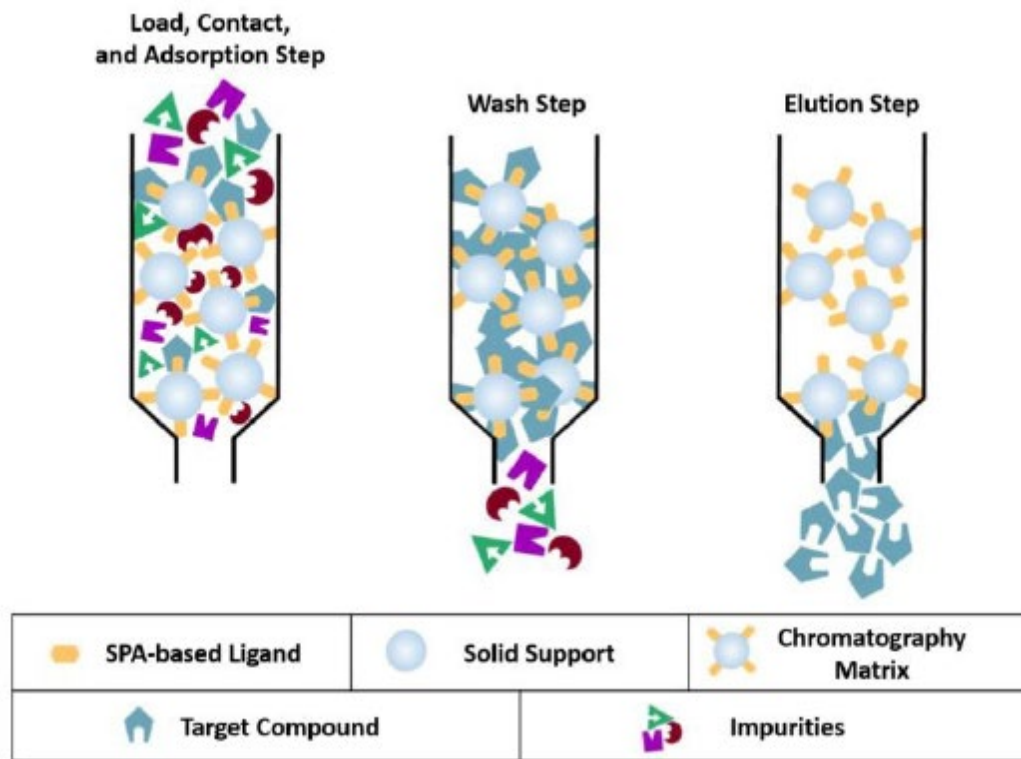
Ex. 2001, 2; *see also* PO Resp. 5.

Below is a generic, exemplary schematic that shows how affinity purification typically works:

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The figure shows the schematic of the loading, contact, and adsorbing step onto a column, followed by the wash step, and finally the elution and collection of the target compound. Ex. 1002 ¶ 24 (citing Ex. 1014 §§ 1.1, 4.2.); *see also* PO Resp. 7 (“In a typical process, the composition containing the desired antibody then is loaded onto (i.e., pumped or injected into) the column.”); Pet. 6; *see generally* Ex. 1014.

F. *The '007 patent (Ex. 1001)*

The '007 patent discloses an affinity ligand useful for isolating antibodies or antibody fragments. *See* Ex. 1001, Abstr., 1:43–49. Affinity ligands were previously used to capture antibodies (immunoglobulin proteins) in chromatography matrices. *See id.* at 2:1–19. After each use, chromatography matrices are cleaned using an alkaline protocol “known as Cleaning In Place (CIP).” *See id.* at 2:20–54. CIP damages protein-based

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affinity ligands through deamidation and cleavage of peptides. *Id.* at 2:35–54.

The '007 discloses prior efforts to genetically engineer protein-based affinity ligands to improve stability to alkaline cleaning, e.g., CIP. *See id.* at 2:53–54; 3:3–55. Specifically, the '007 patent describes a modified affinity ligand based on *Staphylococcus* protein A (“SpA”). SpA was “widely used” as an affinity chromatography ligand due to its ability to bind to antibodies without affecting the antibodies’ ability to bind to antigens. *Id.* at 2:55–68. However, the '007 patent discloses that unmodified SpA required milder cleaning conditions than conventional CIP to prevent damaging the SpA ligand. *Id.* at 3:18–25. Accordingly, the '007 patent describes a need for modifying SpA to improve alkaline resistance while maintaining binding selectivity. *Id.* at 3:25–28.

The '007 patent describes prior efforts to modify SpA through its constituent domains. *See id.* at 3:42–55. SpA is composed of five domains, designated as domains E, D, A, B, and C, which are able to bind to antibodies at the antibodies’ fragment crystallizable (“Fc”) region. *Id.* at 2:60–65. The '007 patent describes a known modified SpA B-domain with “increased chemical stability at pH-values of up to about 13–14.” *Id.* at 3:42–55 (citing WO 03/080655). The increased stability results from mutating “at least one asparagine residue . . . to an amino acid other than glutamine or aspartic acid,” as it was known that asparagine and glutamine residues were sensitive to deamidation and cleavage in alkaline conditions. *See id.* at 2:42–47; 3:42–55.

Against this background, the '007 patent discloses an affinity ligand based on SpA domain C that “is capable of withstanding repeated cleaning-

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in-place cycles.” *Id.* at 4:9–13. The Specification discloses that “Domain C ligand, which contains as many as six asparagine residues, was not . . . expected to present any substantial alkaline-stability as compared to protein A.” *Id.* at 5:54–56. The asparagine residues are shown in the amino acid sequence of wild-type SpA Domain C SEQ ID NO 1. *Id.* at 6:40–43; 15:1–25. The Specification describes “a specific embodiment [of] the chromatography ligand according to the invention [that] comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A.” *Id.* at 6:56–61. In other words, the modified SpA Domain C includes alanine (A) instead of glycine (G) at position 29 of the amino acid sequence. *See id.* at 15:52–54. The ’007 patent further describes a multimeric chromatography ligand including at least two Domain C units, or functional variants thereof. *Id.* at 7:35–38.

1. Illustrative Claims

Claims 1 and 20 are the independent claims, reproduced below, and are illustrative of the claimed subject matter of the ’007 patent.

1. A process for isolating one or more target compound(s), the process comprising:

- (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 52 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G)

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at a position corresponding to position 29 of SEQ ID NO. 1; and

- (b) adsorbing the target compound(s) to the ligand;
- (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand; and,
- (d) performing a cleaning in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.

20. A process for isolating one or more target compound(s), the process comprising:

- (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids in alignment with SEQ ID NO. 1, and wherein each polypeptide has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1;
- (b) adsorbing the target compound(s) to the ligand; and,
- (d) performing a clean in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.

Ex. 1001, 15:40–61, 17:22–40

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II. ANALYSIS

A. *Principles of Law*

“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify “with particularity . . . the evidence that supports the grounds for the challenge to each claim”)). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat’l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner must demonstrate by a preponderance of the evidence³ that the claims are unpatentable. 35 U.S.C. § 316(e); 37 C.F.R. § 42.1(d). A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time of the invention to a person having ordinary skill in the art. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations, including “the scope and content of the prior art”; “differences between the prior art and the claims at issue”; “the level of ordinary skill in the art;” and “objective evidence of non-obviousness.” *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

³ The burden of showing something by a preponderance of the evidence requires the trier of fact to believe that the existence of a fact is more probable than its nonexistence before the trier of fact may find in favor of the party who carries the burden. *Concrete Pipe & Prods. of Cal., Inc. v. Constr. Laborers Pension Tr. for S. Cal.*, 508 U.S. 602, 622 (1993).

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In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. *Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner asserts that a person of ordinary skill in the art would have had

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(1) at least an advanced degree (*e.g.*, a Master's or Ph.D.) in biochemistry, process chemistry, protein chemistry, chemical engineering, molecular and structural biology, biochemical engineering, or similar disciplines; (2) several years of post-graduate training or related experience (including industry experience) in one or more of these areas; and (3) an understanding of the various factors involved in purifying proteins using chromatography.[] Such a person would have had multiple years of experience with affinity ligand design and protein purification.

Pet. 9–10 (citing Ex. 1002 ¶¶ 13–14). Patent Owner does not dispute Petitioner's definition of the person of ordinary skill. *See generally* PO Resp. Because Petitioner's proposed definition is unopposed and appears consistent with the Specification and art of record, we apply it here.

C. *Claim Construction*

The Board applies the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b). 37 C.F.R. § 42.200(b) (2021). Under that standard, claim terms “are generally given their ordinary and customary meaning” as understood by a person of ordinary skill in the art at the time of the invention. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc).

Claims 1 and 20 recite “at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides.” Ex. 1001, 15:46–49; 17:28–31. Petitioner argues that “‘the ligand comprising at least two polypeptides’ refers to a multimeric ligand (such as a tetramer) comprised of multiple polypeptides, each of which is a monomer.” Pet. 19–20 (citing Ex. 1002 ¶¶ 43–45). Petitioner argues that this construction is consistent with Patent

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Owner's implicit construction in the Delaware litigation between the parties.

Id.

Patent Owner does not challenge Petitioner's claim construction. *See generally* PO Resp.

According to the Specification, "the present invention . . . relates to a multimeric chromatography ligand (also denoted a 'multimer') comprised of at least two domain C units, or a functional fragments [sic] or variants thereof." Ex. 1001, 7:35–38. The Specification additionally recites that a multimer containing only domain C units can, however, include linkers. *Id.* at 7:39–41. In addition, the Specification describes that "the multimer comprises one or more additional units, which are different from Domain C." *Id.* at 7:52–53. Based on these disclosures in the Specification, a multimer is composed of at least two or more monomers. Because Petitioner's construction is consistent with the '007 patent's express construction of the term, we apply that construction for the purposes of this decision.

Having considered the parties' positions and evidence of record, we determine that the express construction of any other claim term is unnecessary to resolve the disputed issues in this matter. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))). To the extent further discussion of the meaning of any claim term is necessary to our decision, we provide that discussion below in our analysis of the asserted grounds of unpatentability.

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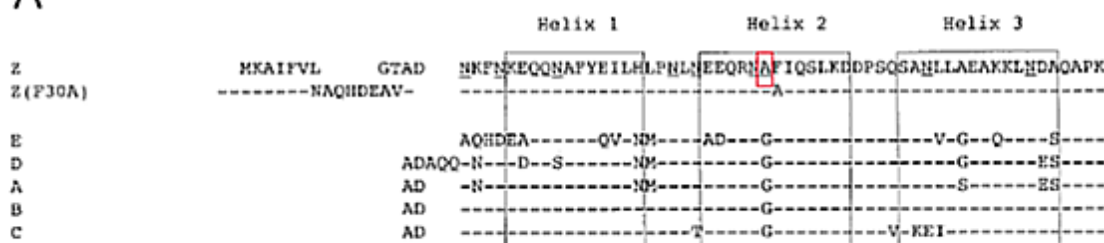
D. *Overview of Asserted References*1. *Linhult (Ex. 1004)*

Linhult is titled “Improving the Tolerance of a Protein A Analogue to Repeated Alkaline Exposures Using a Bypass Mutagenesis Approach.”

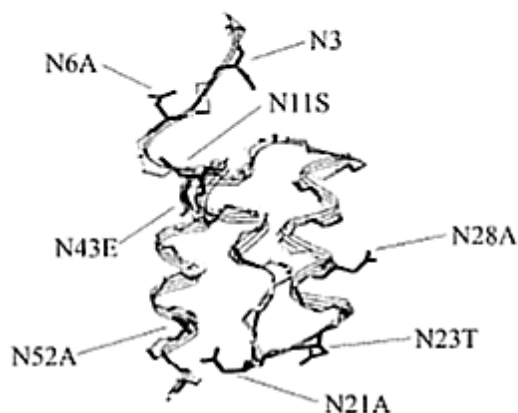
Ex. 1004, 1. Linhult discloses that due to the high affinity and selectivity of Staphylococcal protein A (SPA), “it has a widespread use as an affinity ligand for capture and purification of antibodies” but that “it is desirable to further improve the stability in order to enable an SPA-based affinity medium to withstand even longer exposure to the harsh conditions associated with cleaning-in-place (CIP) procedures.” *Id.*, Abstr. Linhult discloses, “[t]o further increase the alkaline tolerance of SPA, we chose to work with Z, which is a small protein derived from the B domain of SPA.” *Id.* at 2.

Figures 1A and 1B of Linhult are reproduced below.

A



B



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Figure 1A shows “[a]mino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C)” in which the horizontal lines indicate amino acid identity and “one glycine in the B domain [is] replaced [and] underlined” as annotated by the Board with a red box. *Id.* “Z(F30A), and all mutants thereof includes the same N-terminal as Z(F30A)” and “Z(N23T) was constructed with the same N-terminal as Z.” *Id.*⁴ Figure 1B shows “[t]he three-dimensional structure of the Z domain” and “the different substitutions are indicated.” *Id.* Specifically, Linhult discloses,

[t]he B domain has been mutated in order to achieve a purification domain resistant to cleavage by hydroxylamine. An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine.[] Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.[] Protein Z is well characterized and extensively used as both ligand and fusion partner in a variety of affinity chromatography systems.

Id. Using a 0.5 M NaOH cleaning agent and “a total exposure time of 7.5 h for Z(F30A) and mutants thereof,” Linhult determines that “N23 seems to be very important for the functional stability after alkaline treatment of Z(F30A)” and “Z(F30A, N23T) shows only a 28% decrease in capacity despite the destabilizing F30A-mutation.” *Id.* at 410–11; Figs. 2, 3. Linhult reports that “[h]ence, the Z(F30A, N23T) is almost as tolerant as Z and is thereby the most improved variant with Z(F30A) as scaffold.” *Id.* at 411; Figs. 2, 3.

⁴ The mutation N23T having a change in amino acid correlates with the amino acid N next to the “Helix 2” box of Figure 1A as annotated by Petitioner. *See* Pet. 12.

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Linhult further discloses that “Z, Z(F30A), and mutated variants were covalently coupled to HiTrap™ affinity columns,” that “[t]he Z domain includes 8 asparagines (N3, N6, N11, N21, N23, N28, N43, and N52; Fig. 1),” and that “since the amino acid is located outside the structured part of the domain, it will most likely be easily replaceable during a multimerization of the domain to achieve a protein A-like molecule.” *Id.* at 410. Linhult confirms that “the affinity between Z(F30A) and IgG was retained despite the mutation.” *Id.* In Linhult’s studies, “[h]uman polyclonal IgG in TST was prepared and injected onto the columns in excess” and “[a] standard affinity chromatography protocol was followed.” *Id.*

2. *Abrahmsén (Ex. 1005)*

Abrahmsén “relates to a recombinant DNA fragment coding for an immunoglobulin G ([I]gG) binding domain related to staphylococcal protein A . . . and to a process for cleavage of a fused protein expressed by using such fragment or sequence.” Ex. 1005, 1:8–13. Abrahmsén discloses that “[b]y making a gene fusion to staphylococcal protein A any gene product can be purified as a fusion protein to protein A and can thus be purified in a single step using IgG affinity chromatography.” *Id.* at 1:22–26. Abrahmsén explains that Protein A has “5 Asn-Gly in the IgG binding region of protein A” which “makes the second passage through the column irrelevant as the protein A pieces released from the cleavage will not bind to the IgG.” *Id.* at 1:58–63. Abrahmsén provides a solution to this problem “by adapting an IgG binding domain so that no Met and optionally no Asn-Gly is present in the sequence.” *Id.* at 1:64–67.

Abrahmsén discloses that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.”

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Id. at 2:21–23. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén discloses that the recombinant DNA fragment can “cod[e] for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.” *Id.* at 2:32–37. According to Abrahmsén, from a simulation of the Gly to Ala amino acid change in the computer, it was “concluded that this change would not interfere with folding to protein A or binding to IgG.” *Id.* at 5:13–16.

3. *Hober (Ex. 1006)*

Hober “relates to . . . a mutant protein that exhibits improved stability compared to the parental molecule” and “also relates to an affinity separation matrix, wherein a mutant protein according to the invention is used as an affinity ligand.” Ex. 1006, 1. Hober discloses that removal of contaminants from the separation matrix involves “a procedure known as cleaning-in-place (CIP)” but “[f]or many affinity chromatography matrices containing proteinaceous affinity ligands,” the alkaline environment “is a very harsh condition and consequently results in decreased capacities owing to instability of the ligand.” *Id.* at 1–2. According to Hober, stability to alkaline conditions can be engineered into a protein. *Id.* at 2. To improve the stability of a Streptococcal albumin-binding domain (ABD) in alkaline environments, it has been reported to involve the role of peptide conformation in the rate and mechanism of deamidation of asparaginyl residues and that “the shortest deamidation half time have been associated

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with the sequences -asparagine-glycine and -asparagine-serine.” *Id.* at 2.

Further, from a study of a mutant of ABD that was created, it was concluded that “all four asparagine residues can be replaced without any significant effect on structure and function.” *Id.* at 2–3. Hober points out that the staphylococcal protein A (SPA) contains domains capable of binding to the Fc and Fab portions of IgG immunoglobulins from different species and reagents of this protein with their high affinity and selectivity have found a widespread use in the field of biotechnology. *Id.* at 3. Accordingly, “there is a need in this field to obtain protein ligands capable of binding immunoglobulins, especially via the Fc-fragments thereof, which are also tolerant to one or more cleaning procedures using alkaline agents.” *Id.* at 4.

In one embodiment of Hober, a multimer “comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A” in which “asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids” for advantageous structural stability reasons wherein “the glycine residue in position 29 of SEQ ID NO: 1 has also been mutated, preferably to, an alanine residue.” *Id.* at 12. Hober’s SEQ ID NO: 1 and is reproduced below.

```

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
1          5          10          15

Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln
          20          25          30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala
          35          40          45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
50          55

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Id. at SEQUENCE LISTING 1. SEQ ID NO: 1 shows a domain of *Staphylococcus aureus* having Glycine (Gly) as an amino acid at the position 29, as annotated by the Board via red highlighting.

Hober further discloses that its matrix for affinity separation “comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which [in the] protein at least one asparagine residue has been mutated to an amino acid other than glutamine.” *Id.* at 13. For its method of isolating an immunoglobulin, Hober discloses “in a first step, a solution comprising the target compounds, . . . is passed over a separation matrix under conditions allowing adsorption of the target compound to ligands present on said matrix” and “[i]n a next step, a second solution denoted an eluent is passed over the matrix under conditions that provide desorption, i.e. release of the target compound.” *Id.* at 13.

E. *Obviousness in view of Linhult, Abrahmsén, and Hober*

1. *Petitioner’s Contentions*

a) *Claims 1, 2, and 20*

Petitioner contends that “*Linhult* describes the common use of chromatography matrices in the biotechnology field, and, more specifically, SPA-based chromatography matrices to isolate target compounds.” Pet. 16 (citing Ex. 1002 ¶ 83). Petitioner contends that “*Linhult* describes a process whereby a ‘[h]uman polyclonal IgG in TST^[5]’ was prepared and injected onto the columns in excess,’ ‘[a] standard affinity chromatography protocol was followed,’ and ‘eluted material was detected.’” Pet. 17 (citing Ex. 1004,

⁵ TST is a solution containing 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20. Ex. 1004, 4.

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4). Petitioner contends that a person of ordinary skill in the art “would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of loading a liquid comprising the target compound onto the column, thereby allowing the liquid to contact the recited SPA-based chromatography matrix.” *Id.* (citing Ex. 1002 ¶¶ 88–89). In other words, Petitioner contends that the contacting step is a well-known step in the field of affinity chromatography.

Petitioner contends that “[a]dsorbing target compounds to SPA-based ligands coupled to the solid supports was a well-known and conventional feature of SPA-based affinity chromatography.” Pet. 23–24 (citing Ex. 1002 ¶ 122, *see also id.* ¶¶ 120–121; Ex. 1004, 4).

Petitioner contends that “a [person of ordinary skill in the art] would have further understood a ‘standard affinity chromatography protocol’ would involve the well-known and conventional step of eluting target compounds from SPA-based ligands coupled to the solid supports in a chromatography matrix.” Pet. 24 (citing Ex. 1002 ¶ 124, *see also id.* ¶¶ 121–125; Ex. 1004 ¶ 4). Petitioner, therefore contends that it is well-known that a standard affinity chromatography protocol contains three active steps: (1) contacting, (2) adsorbing, and (3) eluting.

Petitioner contends that Linhult teaches a chromatography matrix. Pet. 18. Specifically, Linhult teaches using a HiTrap chromatography affinity column made up of agarose beads that serve as a solid support for “coupling SPA-based ligands.” Pet. 18 (citing Ex. 1004, 4; Ex. 1002 ¶ 98). “*Linhult* discloses that its SPA-based ligands were ‘coupled to’ the solid support agarose beads [] contained in HiTrap™ affinity columns.” *Id.* at 18 (citing Ex. 1004, 4). Petitioner contends that “Linhult discloses that

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‘multimerization’ of SPA monomers is performed to ‘achieve’ an ‘[SPA-]like’ affinity ligand.” *Id.* at 19 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 103–106). Petitioner contends that “Figure 1(a), *Linhult* describes at least 55 amino acids of SPA’s naturally-occurring C domain (i.e., SEQ ID NO. 1).” *Id.* (citing Ex. 1004, 1, Fig. 1(a); *see* Ex. 1005, Fig. 2; Ex. 1006, Fig. 1; Ex. 1008, 639, Fig. 1). Petitioner contends “that all ‘five SPA domains show individual affinity for the Fc-fragment . . . as well as certain Fab-fragments of [antibodies] from most mammalian species.” *Id.* at 20 (citing Ex. 1004, 1).

Petitioner contends that it was “known that individual SPA domains, including the C domain, could be used to construct SPA-based affinity ligands for purifying proteins.” *Id.* at 20 (citing Ex. 1002. ¶¶ 29, 113; Ex. 1004, 1; Ex. 1006, 12; Ex. 1018 ¶ 29; Ex. 1019, 6:25–34). Petitioner contends that *Linhult* teaches a person of ordinary skill in the art “that avoiding the Asn₂₈-Gly₂₉ dipeptide sequence through a G29A mutation, including on the C domain, would yield an SPA-based ligand having increased alkali-stability.” *Id.* at 21 (citing 1002 ¶¶ 112–116; Ex. 1011; Ex. 1012; Ex. 1013). Petitioner acknowledges that “*Linhult* does not expressly disclose a C(G29A)-based SPA ligand,” but asserts that “[r]egardless, it would have been obvious to a [person of ordinary skill in the art] to modify *Linhult* based on the teachings of *Abrahmsén* to incorporate a C(G29A)-based SPA ligand in a chromatography matrix.” *Id.* at 22 (Ex. 1002 ¶¶ 111–120). Petitioner contends “*Abrahmsén* expressly discloses ‘a recombinant DNA coding for **any of** the E D A B C domains of [SPA], wherein the glycine codon(s) in the Asn_[28]-Gly_[29] coding constellation has

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been replaced by an alanine codon.”’ *Id.* at 22 (bracketing in and emphasis in original) (citing Ex. 1005, 2:32–37).

A [person of ordinary skill in the art] would have had good reason to combine the teachings from *Abrahmsén* with *Linhult* because a G29A mutation was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence, i.e., the “most sensitive amino acid sequence to alkaline conditions,” such as those used in CIP. (Ex. 1002 ¶¶ 119, 135; Ex. 1004, [2].) Moreover, a [person of ordinary skill in the art] would have been drawn to a C-domain-based ligand, which, as *Linhult* describes, shows individual affinity for antibodies and already includes 23T (as well as 43E), which it disclosed as providing “remarkably increased” stability. (Ex. 1002 ¶¶ 115-16; Ex. 1004, [1], [8–9].)

Pet. 23.

Applying the teachings of *Abrahmsén* with *Linhult* would have involved merely combining known elements in the field (e.g., a process for isolating one or more target compounds using an affinity chromatography matrix comprising a G29A-containing ligand coupled to a solid support, as in *Linhult*, and a C(G29A)-based amino acid sequence, as in *Abrahmsén*) according to known ligand-construction methods to yield a predictable result[] (e.g., a process for isolating one or more target compounds using the recited affinity chromatography matrix). (Ex. 1002 ¶136.) *See, e.g., KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 415-21 (2007); *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1239-40 (Fed. Cir. 2010).

Pet. 25–26. Petitioner further contends “*Hober*’s disclosure is in the context of SPA-based affinity chromatography utilizing G29A-containing ligands, and, in fact, further confirms that the teachings of *Abrahmsén* are applicable in this context.” Pet. 52 (citing Ex. 1002 ¶¶ 260–275; Ex. 1006, 10–12); Ex. 1006, 12 (“the present multimer also comprises one or more of the E, D, A, B, and C domains of Staphylococcal protein A. . . . for structural stability

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reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to an alanine residue”).

b) Claims 11 and 29

Petitioner contends that “[t]he capability of ‘bind[ing] to the Fab part of an antibody,’ as recited in claims 11 and 29, is an inherent property of the recited C(G29A)-based SPA ligand.” Pet. 30 (citing Ex. 1002 ¶¶ 155–162).

c) Claims 3–10, 12–14, 16–19, 21–28, 30–32, and 34–37

With respect to claims 3–10, 12–14, 16–19, 21–28, 30–32, and 34–37, Petitioner directs our attention to where in the asserted art of record the various limitations of the dependent claims may be found. *See* Pet. 20 n.13, 26–30, 37–41.

2. Patent Owner’s Contentions

Patent Owner argues that the Petition fails to demonstrate that there would have been motivation and a reason to make and use the chromatography matrix as claimed (PO Resp. 17–48); that the Petition has not established that there is a reasonable expectation of success in arriving at the claimed matrix (*id.* at 49–54); that the art teaches away from making the G29A modification (*id.* at 38–44); that the artisan would have been motivated make additional mutations (*id.* at 44–48).

a) Matrix

According to Patent Owner, “Petitioners fail to explain *why* the POSA would have been motivated to select Domain C’s amino acid sequence as the foundation for an engineered SPA ligand with favorable properties.”

PO Resp. 19. Specifically arguing that the obviousness analysis requires the

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prior art be viewed as a whole. PO Resp. 21 (citing *In re Wesslau*, 353 F.2d 238 (CCPA 1965); *In re Enhanced Sec. Rsch., LLC*, 739 F.3d 1347, 1355 (Fed. Cir. 2014); *Impax Lab 'ys Inc. v. Lannett Holdings Inc.*, 893 F.3d 1372, 1379 (Fed. Cir. 2018)). In other words, because obviousness requires considering the prior art as a whole and no one was working on domain C at the time of the invention, Patent Owner asserts, it would not have been obvious to select domain C for further development or genetic modification.

Patent Owner argues that because nobody was working on domain C at the time the invention was filed, therefore, the selection of domain C for further development could not possibly be obvious. *See* PO Resp. 24 (“Reliance on *KSR* also is foreclosed by the evidence that no one in the art was seeking to modify Domain C.”), *see also id.* at 25 (“But no prior art cited by Petitioners singles Domain C out for further development. Ex. 2025 ¶¶ 89–96”), *id.* at 27 (“Dr. Cramer [Petitioner’s expert] himself highlights, it would have been natural for the POSA to further develop the domain—Domain B—that was best understood and for which there was a crystal structure available. Ex. 1002 ¶ 33; Ex. 2015 at 137:20–138:19; Ex. 2017”), *id.* at 28–29 (“The notion that this body of work would lead the POSA to discard the improved ligands the references themselves focus on, and instead start experimenting with mutations to Domain C—strains credulity. Ex. 2025 ¶¶ 92–95”).

According to Patent Owner, neither Linhult nor Abrahmsén supply the motivation to start with domain C. “Linhult focuses exclusively on, and concerns improvements to, the alkaline stability of Domain Z by mutating asparagine residues. *See* Ex. 2025 ¶¶ 64–67, 92.” PO Resp. 30. “Rather than use Domain C, the POSA reviewing Linhult would be motivated to keep

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working with Domain Z, adopting the N23T mutation. Ex. 2025 ¶ 67 & n.3.”

PO Resp. 31. “Neither Abrahmsén itself nor the Petition provide any reason as to why the POSA would have ‘plucked’ Domain C from among the five listed SPA domains. *WBIP[LLC v. Kohler Co.]*, 829 F.3d 1317, 1337 (Fed. Cir. 2016).” PO Resp. 31–32.

b) Reasonable Expectation of Success

Patent Owner argues that “the field of protein engineering is notoriously unpredictable.” PO Resp. 22 (citing Ex. 2025 ¶¶ 50–52). Arguing that “despite their supposed structural similarity, there are a number of differences between the naturally-occurring domains of protein A, including five different amino acids in the sequences of Domain B (with which the industry was quite familiar) and Domain C (which remained virtually ignored as of the priority date).” *Id.* at 22–23 (citing Ex. 2025 ¶ 48).

Protein engineering is a highly complex and unpredictable field and was all the more so as of the priority date more than fifteen years ago. *See, e.g.,* Ex. 2025 ¶¶ 50-52. . . . As amply demonstrated by the effect of the G29A mutation on Domain Z’s Fab-binding ability, even a single amino acid substitution can drastically alter the properties of a protein. Ex. 2025 ¶ 52; Ex. 2015 at 51:15-52:1 ([Dr. Cramer, Petitioner’s expert] agreeing that a single amino acid change can have a significant effect on a ligand’s binding ability), 18:10-12, 73:16-20.

PO Resp. 35–36.

Patent Owner argues that

The Federal Circuit has rejected arguments premised on the notion that a homologous structure renders an invention obvious, particularly given the difficulty and uncertainty in the art as of the priority date. *See, e.g., Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1208 (Fed. Cir. 1991) (holding the use of a monkey gene to probe for a roughly 90 percent “homologous”

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human gene would not have been obvious, particularly given expert testimony that isolating a particular gene would have been “difficult” and the lack of certainty in the endeavor).

PO Resp. 37–38. Specifically, Patent Owner argues that the Fab-binding capability of a ligand could not have been predicted and therefore there is no reasonable expectation of success in using the ligand in a process of purifying a target compound. *See* PO Resp. 49–55.

c) Teaching Away

Patent Owner argues that the prior art would have told the person of ordinary skill in the art to avoid a G29A a modification to domain C.

PO Resp. 39–44. In other words, it’s Patent Owner’s contention that the prior art teaches away from making this modification. “The very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding ‘negligible’ when implemented in Domain B.” PO Resp. 41. Patent Owner argues that a person seeking to improve Fab binding would have avoided a G29A substitution of domain C. PO Resp. 41–44.

d) Additional Modifications

Patent Owner argues that “the prior art would have taught the POSA to make asparagine substitutions, not glycine substitutions, to address alkaline stability concerns.” PO Resp. 44–45. In other words, Patent Owner argues the prior art would have suggested making additional substitutions most notably in the asparagine residues of domain C. *Id.* at 46.

3. Petitioner’s Reply

In response, Petitioner argues that

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Abrahmsén and *Hober* each expressly pointed to a C(G29A) mutation (Ex. 1005, 2:32-37; Ex. 1006, 12), which was known to increase alkali-stability by avoiding the troublesome Asn₂₈-Gly₂₉ dipeptide sequence (*see, e.g.*, Ex. 1004, [2]). As the Board recognized, “*Abrahmsén* provides motivation for making [the G29A] mutation in **any of the IgG binding domains**.” (Decision, 26; *see also* Ex. 1057, 97:3-16 (Dr. Bracewell admitting that *Abrahmsén* discloses a G29A mutation to any of the five domains, including Domain C).)

Reply 2.

A POSA would have reasonably expected success in combining these teachings to achieve the claimed affinity chromatography matrix given the well-known fact that each individual domain, including Domain C, has affinity for antibodies (Ex. 1004, [1]), as well as *Abrahmsén*’s confirmation that G29A “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1057, 99:13-101:21 Ex. 1005, 5:13-16; Ex. 1002 ¶131).

Id. at 3.

Petitioner argues that Patent Owner “has not disputed that *Abrahmsén* disclosed that G29A ‘would not interfere with folding to protein A or binding to IgG.’ (Ex. 1005, 2:32–37, 5:4–16; Ex. 1057, 109:20–110:17.) Nor does it take issue with its own statements in *Hober* that G29A is advantageous for ‘structural stability reasons.’ (Ex. 1006, 12.)” *Id.* at 9. Petitioner contends that Patent Owner’s lack of binding argument is contradicted by “*Abrahmsén* and *Hober*, which make clear that G29A does not affect the ability of an SPA ligand to bind to an antibody. (Ex. 1005, 2:32–37, 5:4–16; Ex. 1006, 12.)” *Id.* at 10.

Petitioner argues that “a POSA would have started with any one of the naturally occurring domains. (Decision, 26-28.) To then increase alkali stability, a POSA would have made the simplest, well-known substitution: G29A. (Section II.A.1–2; Ex. 1061 ¶¶8–15.)” Reply 11.

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Petitioner argues “that Fab-binding was an inherent feature of a C(G29A)-based ligand—which [Patent Owner] does not appear to dispute. (Decision, 34; [Prelim.] Resp., 53–54.) In fact, [Patent Owner] acknowledges that ‘C(G29A)-based SPA ligands retained substantial Fab-binding ability.’ (Resp., 56–57.)” *Id.* at 14.

Petitioner argues that “Fab-binding is not being used [in the Petition] as part of a finding of a motivation to combine; rather, it is an inherent property [of the composition] being claimed. And necessarily present properties do not add patentable weight when they are claimed as limitations. *In re Kubin*, 561 F.3d 1351, 1357 (Fed. Cir. 2009).” Reply 15–16. Petitioner further argues that Patent Owner’s reliance on *Honeywell* is misplaced because “*Honeywell* had to do with an inherent property being used as a teaching in an obviousness analysis; it did not involve a limitation in the challenged claim reciting an inherent property.” Reply 15 (citing *Honeywell Int’l Inc. v. Mexichem Amanco Holding S.A. De C.V.*, 865 F.3d 1348, 1355 (Fed. Cir. 2017); *see also Pernix Ireland Pain v. Alvogen Malta Operations*, 323 F. Supp. 3d 566, 607(D. Del. 2018)).

4. Patent Owner’s Sur-reply

Patent Owner argues that “Petitioners, and the Institution Decision, overlook an important point of consensus between the parties’ experts: the field of protein engineering is notoriously *unpredictable*.” Sur-reply 2. Patent Owner maintains that Petitioner has not identified a motivation to start from Domain C. *Id.* at 3. Patent Owner argues that “Petitioners would have the Board look past the multitude of references teaching a preference for Domains B and Z—including Petitioners’ foundational references—and seize upon fleeting mentions of Domain C.” *Id.* at 7 (citing *In re Wesslau*,

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353 F.2d 238, 241 (C.C.P.A. 1965)”). Patent Owner argues that “[m]ere sequence homology does not make the field predictable, as both experts observe, Ex. 2015 at 51:15-52:1, 56:4-12, 75:12-22, 73:16-20; Ex. 2025 ¶¶ 50-52; Ex. 2049 at 72:1-73:12, and as the vastly different Fab-binding properties of the near-identical Domains B and Z well illustrate, Ex. 2029 at 8.” *Id.* at 8–9.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7–10.” Sur-reply 11.

5. *Analysis*

a) *Claims 1, 2, and 20*

Independent claims 1, 2, and 20 of the ’007 patent are directed to a method of isolating a target compound using a chromatography matrix composition. Ex. 1001, 15:40–64, 17:22–40. The claims recite three active steps: (1) contacting, (2) adsorbing, (3) eluting the target compound from the chromatography matrix, and (4) cleaning the chromatography matrix in place using a NaOH solution. *Id.* Claim 1 further stipulates that the chromatography matrix composition (a solid support) has the following features: a ligand is attached to the matrix and the ligand is made up of at least two polypeptides comprising 52 contiguous amino acids of SEQ ID NO: 1⁶ each having a G29A mutation. Claim 2 depends from claim 1 and

⁶ Wild type amino acid sequence of domain C from *Staphylococcus* protein A (SPA). *See* Ex. 1001, 4:27, 6:35–36, 6:51–52.

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recites “wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1.” *Id.* at 15:62–64; Pet. 20 n.13. Claim 20 is similar to claim 1, except that claim 20 recites “at least 55 amino acids in alignment with SEQ ID NO. 1” instead of “at least 52 contiguous amino acids of modified SEQ ID NO. 1” as recited in claim 1, and does not recite the eluting step. *Id.* at 17:22–40.

(1) *Method*

Claims 1, 2, and 20 are directed to a method of using a particular matrix. The method comprises two parts: (a) the process steps and (b) the structure of the matrix.

(a) *Process Steps*

Petitioner asserts that the combination of Linhult, Abrahmsén, and Hober teaches or suggests the standard affinity chromatography process steps of (1) contacting, (2) adsorbing, (3) eluting, and (4) cleaning in place for the reasons set forth in the Petition. Pet. 16–31; Ex. 1002 ¶ 24.

Linhult teaches making affinity chromatography columns with protein Z, Z(F30A), and other mutated variants. These modified proteins were covalently attached to HiTrap columns in Linhult using NHS-chemistry. Ex. 1004, 4. Linhult uses an affinity matrix column to isolate IgG and measures the loading capacity of the column after repeated cleaning in place (CIP) cycles. Ex. 1004, 4. In Linhult’s studies, human polyclonal IgG was prepared and injected onto the columns in excess and “[a] standard affinity chromatography protocol was followed.” *Id.* at 4. We find that Linhult’s loading of IgG onto the column satisfies the contacting step as recited in the claims. Ex. 1004, 4, *see also id.* (“The columns were pulsed with TST

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(25mM Tris-HCl pH 7.5, 150 mM NaCl, 1.25 mM EDTA, 0.05% Tween 20) and 0.2 M HAc, pH 3.1. Human polyclonal IgG in TST was prepared and injected onto the columns in excess. A standard affinity chromatography protocol was followed for 16 cycles.”). Linhult teaches that “the amount of eluted IgG was measured after each cycle to determine the total capacity of the column.” *Id.* Linhult thereby expressly teaches the contacting and eluting steps, and following standard chromatography protocols the adsorbing step is implied. *Id.*; Ex. 1002 ¶ 24 (“After loading is completed, an additional step to wash out certain remaining impurities is employed. ([Ex. 1006 at 15–17]). Following the loading and wash steps, a different solution, typically one of low pH, is applied onto the column to elute the antibody”). Linhult also teaches a cleaning in place step using an alkaline cleaning agent. “The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min, resulting in a total exposure time of 7.5 h for Z(F30A) and mutants thereof.” Ex. 1004, 4.

Abrahmsén teaches using an IgG bound column for purifying the dimeric Z fragment from a supernatant. Ex. 1005, 9:60–10:15; Ex. 1002 ¶¶ 64–68. Abrahmsén affinity purification protocol is as follows:

The supernatant was passed through the column at a speed of 12 ml/h and the amount of IgG binding material [i.e. the Z fragment] was analyzed before and after it was run through the column. The bound material was washed with TS [(150 mM NaCl 50 mM tris HC pH 7.5)] supplemented with 0.05% Triton X-100 and then TS and finally with 0.05 M ammonium acetate before elution with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.

Ex. 1005, 10:8–16. The contacting step in Abrahmsén’s protocol occurs when the “supernatant was passed through the column,” the adsorbing step occurs before or during the time “[t]he bound material was washed,” and

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“elution” step occurs when the column is treated “with 1M acetic acid pH adjusted to 2.8 with ammonium acetate.” *Id.* Although Abrahmsén column has IgG bound to the column, instead of an SPA domain, the reference still teaches the common chromatography steps of (1) contacting, (2) adsorbing, and (3) eluting a target molecule.

Hober teaches that “protein monomers can be combined-into multimeric proteins, such as dimers, trimers, tetramers, pentamers etc.”

Ex. 1006, 11. These monomer units can be linked with stretches of amino acids ranging from 0–15 amino acids. *Id.* Hober teaches

a matrix for affinity separation, which matrix comprises ligands that comprise immunoglobulin-binding protein coupled to a solid support, in which protein at least one asparagine residue has been mutated to an amino acid other than glutamine. . . The mutated protein ligand is preferably an Fc fragment-binding protein, and can be used for selective binding of IgG. . . the ligands present on the solid support comprising a multimer.

Ex. 1006, 13. Hober describes a typical chromatographic run cycle consisting of: sample application of 10 mg polyclonal human IgG; extensive washing-out of unbound proteins; elution at 1.0 ml/min with elution buffer; followed by Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0.5 M NaOH of 1 hour. *Id.* at 37.

Patent Owner does not dispute that the references disclose the recited chromatography process of contacting, adsorbing, eluting, and cleaning in place. *See generally* PO Resp.; *see id.* at 7 (citing Ex. 1002 ¶ 24; Ex. 2025 ¶ 43).

(b) Chromatography Matrix

The dispute between the parties is whether a person of ordinary skill in the art would have modified the process disclosed in Linhult using the

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G29A modified SPA C domain as disclosed in Abrahmsén. Pet. 22

(“Abrahmsén unequivocally discloses performing a G29A mutation on SPA’s C domain.”); *See* PO Resp. 17–54.

Linhult discloses a process for isolating one or more target compound(s) using chromatography matrices (solid support) comprising SPA ligands. Pet. 16–32. Linhult explains that SPA is a cell surface protein expressed by *Staphylococcus aureus* and consists of five highly homologous domains (E, D, A, B, and C). Ex. 1004, 1. Each of “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” *Id.* (bracketing in original). “Due to the high affinity and selectivity of SPA, it has a widespread use as an affinity ligand for capture and purification of antibodies.” Ex. 1004, Abstr., *see also id.* at 1 (“SPA has a widespread use in the field of biotechnology for affinity chromatography purification, as well as detection of antibodies.”).

Linhult explains that, in column chromatography, sodium hydroxide (NaOH) is probably the most extensively used cleaning agent for removing contaminants such as nucleic acids, lipids, proteins, and microbes, and a CIP step is often integrated in the protein purification protocols using chromatography columns. Ex. 1004, 1. “Unfortunately, protein-based affinity media show high fragility in this extremely harsh environment, making them less attractive in industrial-scale protein purification. SPA, however, is considered relatively stable in alkaline conditions.” *Id.* at 2. Linhult explains that the combination of asparagine with a succeeding glycine is the most sensitive amino acid sequence to alkaline conditions. *Id.* Linhult teaches that “[a]n exchange of glycine 29 for an alanine has been

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made in order to avoid the amino acid combination asparagine–glycine, which is [sensitive to alkaline conditions and is also] a cleavage site for hydroxylamine.” *Id.*

Petitioner’s declarant, Dr. Cramer avers that the “Z” domain referenced in Linhult refers to a synthetic version of the wild-type (i.e., natural) B domain of SPA, in which the naturally occurring glycine in the Asn₂₈-Gly₂₉ dipeptide sequence is replaced by an alanine residue to create an Asn₂₈-Ala₂₉ dipeptide sequence. Ex. 1002 ¶ 30 (citing Ex. 1004, 2, Fig. 1(a); Ex. 1007, 3, Fig. 1); ¶ 31 (citing Ex. 1005). We credit Petitioner’s declarant, Dr. Cramer for establishing that the C domain sequence disclosed in Linhult contains 55 amino acids in SEQ ID NO: 1 as claimed. Ex. 1002 ¶ 109 (showing a sequence alignment), *see also id.* ¶ 322 (showing sequence alignment of domain C of Abrahmsén with SEQ ID NO: 1).

According to Abrahmsén, the IgG binding domains E, D, A, B, and C of SPA were known. *See* Ex. 1005, 3:25–35, 4:34–37, Fig. 2. Relying on “computer analysis [Abrahmsén] surprisingly showed that the Gly in the Asn-Gly dipeptide sequence could be changed to an Ala. This change was not obvious as glycines are among the most conserved amino acids between homologous protein sequences due to their special features.” *Id.* at 5:7–9. Abrahmsén teaches that in a preferred embodiment, “the glycine codon in the Asn-Gly constellation has been replaced by an alanine codon.” *Id.* at 2:21–23. Thus, Abrahmsén provides motivation for making this mutation in any of the IgG binding domains E, D, A, B, and C of Staphylococcal protein A. Abrahmsén teaches recombinant DNA fragments coding for any of the E, D, A, B, and C domains of Staphylococcal protein A, wherein the glycine

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codon(s) in the Asn-Gly coding constellation have been replaced by an alanine codon. *Id.* at 2:33–37.

Abrahmsén, like Linhult, exemplifies the cloning of and expression of the Z-fragment. Ex. 1005, 7:65–10:56. Abrahmsén teaches that “the Z-region is the part of the Z-fragment coding for the IgG binding domain.” *Id.* at 3:39–41. Abrahmsén purifies the recombinant Z protein using an IgG column. *Id.* at 10:26–28. In one embodiment, Abrahmsén provides “a recombinant DNA sequence comprising at least two Z-fragments” in which “the number of such amalgamated Z-fragments is preferably within the range 2–15, and particularly within the range 2–10.” *Id.* at 2:27–31. Abrahmsén, therefore, reasonably suggests making multimeric constructs. Abrahmsén also uses column chromatography to purify a Z domain containing protein.

Hober teaches a multimer ligand that

also comprises one or more of the E, D, A, B, and C domains of *Staphylococcal* protein A. In this embodiment, it is preferred that asparagine residues located in loop regions have been mutated to more hydrolysis-stable amino acids. In an embodiment advantageous for structural stability reasons, the glycine residue in position 29 of SEQ ID NOS. 1 has also been mutated, preferably to, an alanine residue. Also, it is advantageous for the structural stability to avoid mutation of the asparagine residue in position 52, since it has been found to contribute to the α -helical secondary structure content of the protein A molecule.

Ex. 1006, 12, *see also id.* at 9 (“SEQ ID NO 1 defines the amino acid sequence of the B-domain of SpA”).

Here, the teachings of Linhult, Abrahmsén, and Hober suggest mutating the glycine at position 29 for an alanine in any one of the IgG binding domains of E D A B or C of SPA in order to avoid protein

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degradation. Ex. 1004, 2; Ex. 1005, 5:4–9. We, therefore, agree with Petitioner that the art expressly suggests that the glycine codon can be mutated for an alanine codon in any one of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 99–111). Attaching any one of SPA mutated IgG binding domains E D A B or C to a matrix using “known ligand-construction methods to yield a predictable result[] (e.g., the claimed affinity chromatography matrix)” would have been obvious. Pet. 25 (citing Ex. 1002 ¶ 130). As the Federal Circuit has explained, “[w]here a skilled artisan merely pursues ‘known options’ from ‘a finite number of identified, predictable solutions,’ the resulting invention is obvious under Section 103.” *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1070 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421).

Accordingly, we agree with Petitioner that the combination of Linhult Abrahmsén, and Hober expressly suggests mutating the glycine codon for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Pet. 23 (citing Ex. 1002 ¶¶ 115–116). We also agree that the cited art teaches using these mutant SPA domains in column chromatography for the isolation of antibodies.

We address Patent Owner’s contentions below.

(2) *Response*

(a) *Matrix*

We do not find Patent Owner’s argument that the Petition fails to identify a reason to select domain C persuasive. PO Resp. 17–38. Specifically, we are not persuaded by Patent Owner’s contention that just because nobody was working on domain C at the time the invention was filed means selection of domain C would not have been obvious. *See* PO

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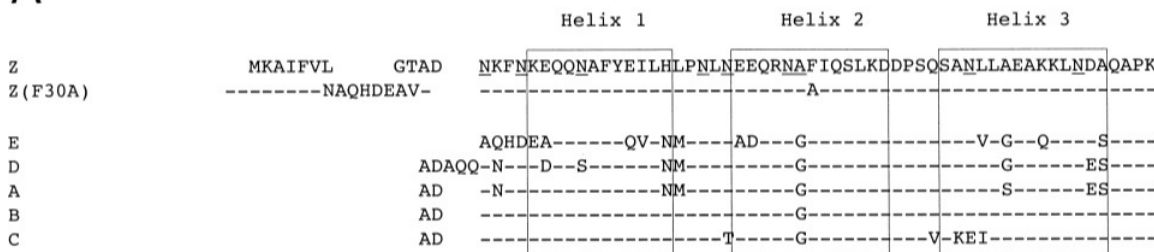
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Resp. 32 (“A general recognition that there exist five naturally occurring protein A domains is not a motivation to use each of them as a starting point for the claimed mutations”).

Petitioner’s articulated obviousness ground is premised on the knowledge that *any one* of the five SPA IgG binding domains are known to bind IgG and can function as a ligand for the purification of antibodies. Linhult and Abrahmsén both expressly suggest that the glycine codon at position 29 can be mutated for an alanine codon in *any one* of the SPA IgG binding domains E, D, A, B, or C. Ex. 1004, 2; Ex. 1005, 2:32–37. Here, the SPA IgG binding domains comprise a short list of 5 members: E, D, A, B, or C. Of these 5 members, the glycine at position 29 in domain B has already been mutated to an alanine to create a domain Z which has been shown to retain IgG binding activity. Ex. 1004, 6 (Fig. 3). Figure 1A of Linhult is reproduced below.

A



Linhult’s Figure 1A, reproduced above, shows the amino acid alignments of the Z, Z(F30A) and the five homologous domains (E, D, A, B, and C). The three boxes show the α -helices. Ex. 1004, 2; Ex. 1005, Fig. 2.

Here, Linhult and Abrahmsén show that the IgG binding domains of SPA– E, D, A, B, or C share many structural similarities. See Ex. 1004, 2 (Fig. 1(a) (reproduced above)); Ex. 1005, 3:25–35. As discussed in our Institution Decision (Dec. 30–31), there are a finite number – five (5) – SPA

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IgG binding domains and each possesses the dipeptide sequence Asp-Gly known to be a target for alkaline protein degradation. Therefore, the solution of mutating the glycine at position 29 for an alanine to remove the alkaline sensitive sequence is not a product of innovation but of ordinary skill and common sense. *See Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1364-65 (Fed. Cir. 2012) (quoting *KSR*, 550 U.S. at 421). It is well established that

[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties.

In re Deuel, 51 F.3d 1552, 1558 (Fed. Cir. 1995).

There is also an express teaching in both Linhult and Abrahmsén to mutate the glycine at position 29 to an alanine in order to prevent degradation of the protein and increase stability, which further supports the obviousness of incorporating the mutation into any one of the IgG binding domains that has the Asn-Gly dipeptide. *See, e.g., SIBIA Neurosciences, Inc. v. Cadus Pharm. Corp.*, 225 F.3d 1349, 1358–59 (Fed. Cir. 2000) (stating that an express teaching in the prior art suggesting a particular modification establishes obviousness).

Because the G29A modification would have provided ligands that are less susceptible to alkaline conditions and are resistant to hydroxylamine cleavage, Petitioner has provided a sufficient evidence-backed reason for making the modification in any one of the domains. Pet. 16–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

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(b) Reasonable Expectation of Success

We are not persuaded by Patent Owner’s contention that there is no reasonable expectation of success in using a G29A mutation in domain C. PO Resp. 49–55.

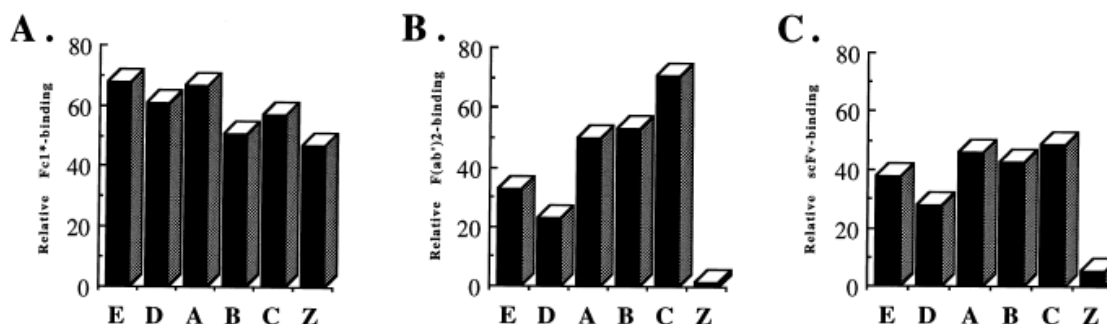
Linhult explains that removing the Asp–Gly amino acid combination not only results in the removal of the hydroxylamine cleavage site but also creates a product that is more alkaline resistant. *See* Ex. 1004, 2 (“An exchange of glycine 29 for an alanine has been made in order to avoid the amino acid combination asparagine–glycine, which is a cleavage site for hydroxylamine. Asparagine with a succeeding glycine has also been found to be the most sensitive amino acid sequence to alkaline conditions.”).

Abrahmsén teaches that this Asn-Gly amino acid combination is present in all five SPA IgG binding domains and that mutating the dipeptide would not interfere with IgG binding. Ex. 1005, 4:56–58 (“The Asn-Gly dipeptide sequence is sensitive to hydroxylamine. As this sequence is kept intact in all five IgG binding domains of protein A. . . . However, by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”). Abrahmsén’s conclusion that the mutation would not interfere with binding to IgG is supported by Abrahmsén (*see* Ex. 1005, 9:60–10:35), Linhult (*see* Ex. 1004, 6 (Fig. 3)), and Jansson (Ex. 2029).⁷ Jansson’s Fig. 3,

⁷ Patent Owner cites Jansson (Ex. 2029) for the position that domain Z has negligible binding to Fab. *See* PO Resp. 40–41. However, claims 1, 2, and 20 are not limited to Fab binding. Indeed the claims do not even require IgG binding.

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reproduced below.



Jansson Figure 3 (Panel A), reproduced above, shows a side-by-side comparison of Fc1*, Fab, and scFv binding to SPA domains. The figure shows that the single G29A mutation between domain B and domain Z results in a protein that is able to bind IgG.⁸ Comparing panel A - domain B domain with panel A - domain Z, the relative binding remains close to 50%, indicating that the G29A mutation between domains B and C does not interfere with IgG binding. Ex. 2029, 6. This is a result predicted by Abrahmsén's computer modeling and substantiated by Abrahmsén domain Z purification and Linhult's IgG purification. *See* Ex. 1005, 4:56–58, 9:60–10:35; Ex. 1004, 6 (Fig. 3).

“Obviousness does not require absolute predictability of success . . . all that is required is a reasonable expectation of success.” *In re Droge*, 695 F.3d 1334, 1338 (Fed. Cir. 2012) (quoting *In re Kubin*, 561 F.3d 1351, 1360

⁸ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the “IgG control” in Jansson. Patent Owner's counsel explains that “Part A is Fc binding. So that is, I believe the way they did this experiment was with Fc fragments, but it's generally acknowledged, you know, these antibodies all have an Fc domain if they're a whole antibody and that reflects the fact that all of these domains A, B, C, D and E and domain Z, which is B with the G29A mutation, retain this Fc binding.” Tr. 70:6–11.

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(Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894, 903–04 (Fed. Cir. 1988)); *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016) (explaining that the expectation of success issue involves a showing of “a reasonable expectation of achieving *what is claimed*”) (emphasis added). “Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention.” *Pharma Stem Therapeutics, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1363–1364 (Fed. Cir. 2007).

Here, the record supports that each individual SPA domain, including the C domain, has affinity for IgG antibodies. Ex. 1004, 1 (“The five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” (bracketing in original)). Abrahmsén suggests making a mutation of Asn-Gly coding constellation in *any one* of the SPA domains by replacing a glycine codon with an alanine codon to remove the Asn-Gly dipeptide sequence known to be sensitive to hydroxylamine degradation. *See* Ex. 1005, 4:56–5:16, *see also id.* Fig. 2 (showing the Asn-Gly coding constellation in all SPA domains); Ex. 1006, 2 (“the shortest deamidation half times have been associated with the sequences –asparagine–glycine and – asparagine–serine”). Abrahmsén’s confirms that a G29A mutation on SPA would not interfere with folding of SPA protein and the binding to antibodies. Ex. 1005, 5:13–16 (“by simulating the Gly to Ala amino acid change in the computer we concluded that this change would not interfere with folding to protein A or binding to IgG.”), 9:60–10:35 (using IgG columns to purify protein Z dimers). Abrahmsén’s computer modeling suggests that IgG binding is not impacted

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by the mutation and this is confirmed by Linhult's experiments showing that the G29A mutant of domain B (a.k.a. domain Z) binds IgG. Ex. 1004, 6 (Fig. 3); *see also* Ex. 1005, 9:60–10:35.

Patent Owner argues that “Abrahmsén’s computer simulation was of unmodified Protein A as a whole, not a Domain C (or G29A-modified) monomer or multimer, and thus does not reveal the impact of a G29A mutation on protein folding or IgG affinity. Ex. 2025 ¶ 103; Ex. 2049 at 131:7-10.” Sur-reply 11.

We are not persuaded by Patent Owner’s contention that the information gained by computer modeling of the SPA native domain B – IgG crystal structure could not be extrapolated to other SPA domains that are structurally very similar.

As Petitioner’s expert, Dr. Cramer explains

[i]t was well known that the researchers who developed the Z domain based on the wild-type B domain (rather than any of the other four SPA domains) did so for two reasons. (*See, e.g.*, Ex. 1007 at 109.) First, a crystal structure of the wild-type B domain binding to an antibody happened to be available in 1981 for analysis. (*See, e.g., id.*; Ex. 1005 at col. 4:56-68; Ex. 1017.) And, second, *their work would be informative of mutations that could be done on all five of the highly homologous SPA domains more generally.* (*See, e.g.*, Ex. 1005 at col. 2:32-37; Ex. 1007 at 109; Ex. 1008 at 639, Fig. 1.)

Ex. 1002 ¶ 33 (emphasis added). Dr. Cramer further explains that “[t]hey did the computer modeling based on that complex because that’s the crystal structure that they had. It wasn’t done because the B domain is special. . . . And then there’s several other places where they state clearly that they could also do the other domains with expected similar results.” Ex. 2015, 138:8–22.

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We agree with Petitioner that the teachings of Linhult, Abrahmsén, and Hober provide a reasonable expectation of success at arriving at a chromatography composition that contains the SPA domain C ligand with the G29A mutation that can be used in a process for purifying IgG. Pet. 24 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 124–126), *see id.* at 48–49 (citing Ex. 1006, 10–12; Ex. 1002 236–263).

(c) No Teaching Away

We are also not persuaded by Patent Owner’s contention that the art teaches away from the G29A substitution because it interferes with Fab binding. *See* PO Resp. 39–42; Sur-reply 9; Ex. 2009 at 2; Ex. 2010 at 25; Ex. 2012 at 25–26; Ex. 2029 at 7.

None of claims 1, 2, or 20 recite a need to bind the Fab region of an antibody or that the target molecule is Fab. All that is required by these claims is that they adsorb a target molecule and that you can elute the target molecule from the matrix. The target molecule, therefore, can reasonably encompass IgG.

Petitioner’s articulated rationale is that there was an expectation that the composition binds antibodies, including monoclonal antibodies, and therefore, would be useful in a process of isolating antibodies. Petitioner contends that:

A POSA would have also reasonably expected such a combination to achieve a process for isolating one or more target compounds using the recited affinity chromatography matrix given the well-known fact that each individual SPA domain, including the C domain, has affinity for antibodies (Ex. 1004, [1]) as well as *Abrahmsén*’s confirmation that a G29A mutation on SPA “would not interfere with folding [of SPA] or binding to [antibodies]” (Ex. 1005, 5:13-16). (Ex. 1002 ¶137.)

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Pet. 26; *see also* Reply 8 (“A POSA would not have been motivated only by Fab-binding ability, as even Dr. Bracewell agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’ (Ex. 1057, 75:17-76:4, 113:23-114:11, 157:24-158:9; Ex. 1061 ¶29)”).

The law does not require that the teachings of the reference be combined for the reason or advantage contemplated by the inventor, as long as some suggestion to combine the elements is provided by the prior art as a whole. *In re Beattie*, 974 F.2d 1309, 1312 (Fed. Cir. 1992); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976); *see In re Kemps*, 97 F.3d 1427, 1430 (Fed. Cir. 1996) (“[T]he motivation in the prior art to combine the references does not have to be identical to that of the applicant to establish obviousness.”).

Here, Linhult teaches that “[t]he five SPA domains show individual affinity for the Fc-fragment [11 residues of helices 1 and 2 (domain B)], as well as certain Fab-fragments of immunoglobulin G (IgG) from most mammalian species.” Ex. 1004, 1 (bracketing in original) (citation omitted). Linhult, therefore, teaches that *any one* of the SPA IgG binding domains E, D, A, B, or C can bind the Fc region of an antibody and can therefore be used as a ligand for purifying IgG antibodies. In addition, the combination of Linhult and Abrahmsén suggests making the G29A mutation in each of the domains because it would provide ligands that are less susceptible to protein degradation. Ex. 1004, 2; *see also* Ex. 1005, 2:33-37 (“[A] recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation has been replaced by an alanine codon.”).

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Patent Owner contends that the G29A mutation would lead to a reduction in the Fab binding of domain C, and therefore, would lead away from making the mutation. PO Resp. 40–41 (citing Ex. 2010, 2; Ex. 2011, 25; Ex. 2012, 25–26; Ex. 2013, 2–3; Ex. 2025 ¶¶ 105–109; Ex. 2029, 6–7). Patent Owner’s cited references are directed to Fab binding. But claims 1, 2, and 20 are not limited to Fab binding. Showing that the G29A mutation interferes with Fab binding does not teach away from mutating any of SPA domains E, D, A, B, or C in order to create an IgG binding ligand that retains its’ binding affinity for Fc yet is less sensitive to cleaning in place solutions. *See, e.g.*, Ex. 2013, 3 (“The site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ⁹ binding.”). Accordingly, we are not persuaded by Patent Owner’s contention that with respect to claims 1, 2, and 20 that the art teaches away from Petitioner’s proposed combination.

(d) Additional Modifications

We also disagree with Patent Owner’s contention that the ordinary artisan would not stop with a single G29A mutation in a SPA domain. *See* PO Resp. 44–48. Here, Abrahmsén expressly suggests making only a single mutation. Specifically, Abrahmsén contemplates “a recombinant DNA fragment coding for any of the E D A B C domains of staphylococcal protein A, wherein the glycine codon(s) in the Asn-Gly coding constellation

⁹ Fcγ is the constant region of IgG involved in effector function. Specifically, “[t]he Fcγ binding site has been localized to the elbow region at the CH2 and CH3 interface of most IgG subclasses, and this binding property has been extensively used for the labeling and purification of antibodies.” Ex. 2013, 1. In other words, Fcγ and Fc terminology are used interchangeably in the art.

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has been replaced by an alanine codon” without additional mutations.

Ex. 1005, 2:33–37.

(3) *Summary*

We find that Petitioner has shown by a preponderance of the evidence that the combined teachings of at least Linhult and Abrahmsén suggests the use of *any one* of the SPA IgG binding domains E, D, A, B, or C as the starting ligand for purifying IgG antibodies, and that making the G29A mutation in *any one* of the domains would have been obvious because it would have provided ligands that are less susceptible to alkaline conditions and are resistant to hydroxylamine cleavage. Pet. 16–25; Reply 3–5; Ex. 1061 ¶¶ 8–11; Ex. 1004, 2; Ex. 1005, 2:32–37.

Having considered the evidence and argument cited in the Petition, which we have described above and find persuasive, we are persuaded that Petitioner has shown by a preponderance of evidence of record that the combination of Linhult, Abrahmsén, and Hober teaches each of the limitations of claims 1, 2, and 20. Petitioner not only has articulated a sufficient motivation for making the combination but has also established that there is a reasonable expectation of success for the binding of an IgG antibody to a SPA domain that contains an G29A mutation.

b) Claims 11 and 29

Petitioner argues that “[t]he ‘capab[ility] of binding to the Fab part of an antibody,’ as recited in claims 11 and 29, is an inherent property of the recited C(G29A)-based SPA ligand.” Pet. 30 (citing Ex. 1002 ¶¶ 155–162). Petitioner contends that a person of ordinary skill in the art did not need to recognize the Fab binding property of Domain C to be motivated to select

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that domain for modification. “A POSA would not have been motivated only by Fab-binding ability,[] as even Dr. Bracewell [Patent Owner’s expert] agreed that ‘a POSA would have understood that it was desirable to purify *monoclonal antibodies* for therapeutic use in 2006.’” Reply 8 (citing Ex. 1057, 75:17–76:4, 113:23–114:11, 157:24–158:9; Ex. 1061 ¶ 29).

Patent Owner argues that

[t]he very G29A amino acid substitution Petitioners now suggest the POSA would seek to employ with Domain C would have been known to have rendered Fab binding “negligible” when implemented in Domain B. Ex. 2009 at 2; *see also, e.g.*, Ex. 2010 at 2 (“Fab binding activity is located to a region determined by helices 2-3, including the position mutated to yield the Z domain.”); Ex. 2011 at 25 (“[I]t only takes a single residue change in SpA to eliminate either Fab or Fc binding. The sole difference in domain Z compared to domain B is the substitution of a glycine to an alanine”); Ex. 2012 at 25-26 (“[D]omain Z containing a single G29A-substitution compared to domain B exhibits little or no [Fab] binding. This might be due to the substitution since the C_β of the alanine would perturb the interaction between the two molecules.”).

PO Resp. 41.

Because claim 11¹⁰ is directed to a “[a] process for isolating one or more target compound(s)” and identifies that the target compound is “the Fab part of an antibody” (Ex. 1001, 16:66–67) the evidence needs to show a reasonable expectation that a mutated SPA ligand binds Fab. Without such a showing, there is no reasonable expectation that the process would result in the purification of a Fab target. In other words, because the claims are process claims the Petitioner needs to establish that a mutated SPA domain would reasonably bind a Fab fragment.

¹⁰ Claim 11 and 29 recite similar limitations.

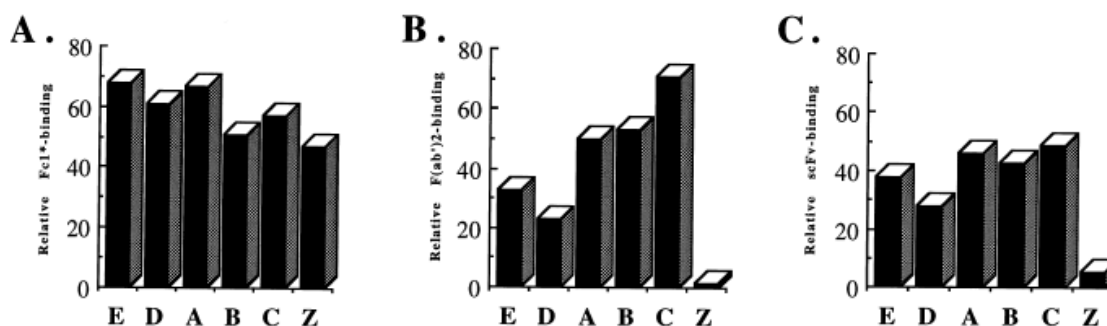
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We agree with Petitioner, that Linhult establishes that a G29A mutation in domain B (resulting in domain Z) does not interfere with IgG binding. *See* Ex. 1004, 6 (Fig. 3 (showing IgG binding with domain Z)). Linhult, however, is silent with respect to Domain Z's ability to bind to Fab fragments. *See generally* Ex. 1004. Abrahmsén similarly establishes Domain Z binding to IgG but is also silent with respect to Domain Z binding Fab. *See generally* Ex. 1005. Hober also does not disclose Fab binding of a mutant SPA domain. *See generally* Ex. 1006. Thus, each of Linhult, Abrahmsén, or Hober are silent with respect to Fab binding to a mutated SPA domain.

Jansson (Ex. 2029), cited by Patent Owner, supports the position that Fab binding to mutated SPA domains is unpredictable. Jansson, just like Linhult, recognizes that “[a]ll [SPA] domains bound to a recombinant human IgG1 Fc fragment with similar strength. For the first time, binding to human Fab was demonstrated for all *native SPA domains*, using both polyclonal F(ab')₂ and a recombinant scFv fragment as reagents.” Ex. 2029, Abstract (emphasis added). Jansson, however, establishes that “the engineered Z domain showed a considerably lower affinity for Fab as compared to the native domains.” *Id.* Jansson Fig. 3, reproduced below, shows that the G29A mutation results in a loss of Fab binding ability.



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Jansson Figure 3, reproduced above, shows the side-by-side comparison of Fc1*¹¹, Fab, and scFv binding and confirms what was already suggested in Linhult, Abrahmsén, and Hober – that a composition containing the G29A mutation in a SPA domain can bind IgG. Ex. 2029, 6 (Fig. 3 (*compare* Panel A-domain B, *with* Panel A-domain Z). Panel B in Jansson Figure 3, however, shows that the single G29A mutation between Domain B and Domain Z results in the loss of Fab binding. Ex. 2029, 6 (Fig. 3 (*compare* Panel B-Domain B, *with* Panel B-Domain Z). At the time the invention was made it was also known that “[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding.” Ex. 2013, 3. Thus, on this record, establishing that a mutation that does not interfere with IgG binding says nothing about the ability of a mutated SPA domain to bind Fab.

We, therefore, agree with Patent Owner’s contention that based on the prior art, the Fab binding capacity was unknown with the modification as suggest by the combination of Linhult, Abrahmsén, and Hober.¹² Patent Owner has provided evidence that Fab biding capacity of a mutated SPA

¹¹ Fc1* is the constant region of human IgG1. Ex. 2029, 4. Fc1* is understood to be used as the IgG control in Jansson. Fc1* is functionally equivalent to Fcγ in SPA binding.

¹² In *JSR Corporation et al. v. Cytiva Bioprocess R&D AB et al.*, IPR2022-00036, Paper 41 at 44–49 (PTAB April 19) (Final Written Decision) we determined that the capability of SEQ ID NO:1 to bind Fab is an inherent feature of the structure claimed. The present claims, however, are directed to a method of isolating Fab which requires prior knowledge that the ligand binds Fab. Fab is a digestion product of a whole IgG molecule treated with papain and is not naturally found in IgG samples. *See above* I.F. In other words, when isolating IgG with a column containing SEQ ID NO: 1 there would be no elution of Fab because the fragments are not present in an IgG containing sample.

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domain protein is unpredictable. *See* PO Resp. 56–57 (Ex. 2029, 5–6). The disclosures in the prior art, therefore, support Patent Owner’s position that “the SPA ligands of the claimed chromatography matrices unexpectedly retained their ability to bind to the Fab part of an antibody despite the substitution of an alanine for the glycine at position 29 of the Domain C sequence.” PO Resp. 57 (citing Ex. 2025 ¶ 123; Ex. 2030, 18–19).

Because the art does not support the conclusion that G29A mutation in a SPA domain ligand binds Fab, Petitioner has not established by a preponderance of the evidence of record that the process of isolating Fab target using a mutated SPA domain C ligand as required by claims 11 and 29 would have been obvious based on the combined teachings of Linhult, Abrahmsén, and Hober.

c) Claims 3–8 and 21–26

Claims 3–8 depend either directly or indirectly from claim 1, and claims 21–26 depend either directly or indirectly from claim 20. These claims further limit the recited processes of claims 1 and 20 whereby the recited matrix retains a percentage of its original binding capacity after certain NaOH exposures.

We are not persuaded by Patent Owner’s argument that “none of Petitioners’ cited references actually describe a C(G29A)-based SPA ligand, let alone provide alkaline stability data or test results concerning the same, the POSA is simply left to guess at how such a ligand would perform.” PO Resp. 50 (citing Ex. 2025 ¶¶ 121–124).

For the reasons discussed above (II.E.5.a), we find that Petitioner has shown by a preponderance of evidence that there is a reason to make the G29A mutation in any one of the SPA IgG binding domains, including

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domain C, and that the use of the mutated protein would reasonably result in a matrix that can be used to purify IgG. Linhult teaches a CIP protocol with at 0.1 to 0.5M NaOH was a well-known and conventional technique. Pet. 30 (citing Ex. 1004, 1–2, 4–5; Ex. 1002 ¶ 152); Ex. 1004, 6 (“Figure 3, the Z(N23T) mutant shows higher resistance to alkaline conditions than the Z domain when exposed to high pH values.”); *see also* Ex. 1006, 39 (“Cleaning-In-Place (CIP) with CIP-buffer with a contact time between column matrix and 0,5 M NaOH of 1 hour”). “Between each cycle [in Linhult], a CIP-step was integrated. The cleaning agent was 0.5 M NaOH and the contact time for each pulse was 30 min.” Ex. 1004, 4. “After 16 [CIP] cycles, giving a total exposure time of 7.5 h, the column with the Z(F30A)-matrix shows a 70% decrease in capacity.” Ex. 1004, 5; *see also* Ex. 1006, 39 (“Each cycle [in Hober] was repeated 21 times resulting in a total exposure time between the matrix and the sodium hydroxide of 20 hours for each different matrix”). Both Linhult and Hober recognize that repeated exposures of a SPA chromatography ligand leads to a reduction of the binding capacity over time. That Linhult recognizes that additional mutations could further improve alkaline stability does not detract from Linhult’s teaching that a composition containing the single G29A mutation in SPA domain B retains IgG binding. Ex. 1004, 6, *see id.* at 4 (“The Z-domain already possesses a significant tolerance to alkaline conditions.”).

Petitioner has shown by a preponderance of the evidence of record that there is a reason for making the G29A mutation in *any one* of the four remaining SPA domains in order to produce a SPA product that is more alkaline stable and would reasonably bind IgG. *See* Pet. 26–28, 30; Reply 20–21; *see* Ex. 1002 ¶¶ 115–119.

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d) Claims 9, 10, 12–14, 16–19, 27–28, 30–32 and 34–37

Petitioner asserts that Linhult, Abrahmsén, and Hober teaches the additional limitations of the dependent claims. *See* Pet. 26–30, 37–41. In support of these arguments, Petitioner directs our attention to the relevant disclosures of Linhult, Abrahmsén, and Hober and provides a detailed claim analysis addressing how each element of the challenged claims are disclosed by the cited prior art and provides explanation as to why one of ordinary skill in the art would have combined the references to arrive at the claimed subject matter with a reasonable expectation of success. *Id.*

Patent Owner does not offer arguments addressing Petitioner’s substantive showing with respect to claims 9, 10, 12–14, 16–19, 27–28, 30–32 and 34–37 separate from its arguments about claims 1, 2, and 20. *See generally* PO Resp.

We have reviewed Petitioner’s arguments and the underlying evidence cited in support, which we adopt as our own, and determine that Petitioner establishes that the combination of Linhult, Abrahmsén, and Hober teaches the additional limitations of these dependent claims. *See e.g.*, Pet. 26 (citing Ex. 1004, 4; Ex. 1002 ¶¶ 112–114), *see id.* at 30 (citing Ex. 1002 ¶¶ 128–129); Ex. 1004, 4 (“a multimerization of the domain to achieve a protein A-like molecule”); Ex. 1005, 9:15–10:35. In particular, we note that Hober teaches that monomeric mutant proteins can be combined into multimeric proteins, such as dimers, trimers, tetramers, pentamers, and other multimers. Ex. 1006, 11. Hober also discloses that the multimer comprises mutant monomer units “linked by a stretch of amino acids preferably ranging from 0 to 15 amino acids, such as 5–10.” *Id.* Petitioner also provides sufficient explanation as to why one of ordinary skill in the art would have combined

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the references to arrive at the claimed subject matter with a reasonable expectation of success. Pet. 26–30, 37–41.

Accordingly, we determine that the preponderance of evidence of record supports Petitioner’s contentions with respect to claims 9, 10, 12–14, 16–19, 27–28, 30–32 and 34–37.

6. *Summary*

For the foregoing reasons, we determine that Petitioner has shown by a preponderance of evidence that of claims 1–10, 12–14, 16–28, 30–32, and 34–37 of the ’007 patent are unpatentable based on the combination of Linhult, Abrahmsén, and Hober.

For the reasons discussed above, Petitioner has not shown by a preponderance of evidence that of claims 11 and 29 of the ’007 patent are unpatentable.

F. *Other Asserted Grounds*

1. *The ’042 IPR Grounds Based on Other Various Combinations of Linhult, Abrahmsén, and Hober*

Petitioner also asserts that claims 1–11 and 20–29 are unpatentable as obvious over Linhult and Abrahmsén (Pet. 18–30); that claims 1–14, 16–32, and 34–37 are unpatentable as obvious over Linhult and Hober (*id.* at 30–48); and that claims 1–14, 16–32, and 34–37 are unpatentable as obvious over Abrahmsén and Hober (*id.* at 49–60) under 35 U.S.C. §103(a).

Because Petitioner has already shown that challenged claims 1–10, 12–14, 16–28, 30–32, and 34–37 are unpatentable over Linhult, Abrahmsén, and Hober as obvious, as discussed *supra*, we do not reach these claims in these additional asserted grounds as to those claims. *See Beloit Corp. v.*

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Valmet Oy, 742 F.2d 1421, 1423 (Fed. Cir. 1984) (“The Commission . . . is at perfect liberty to reach a ‘no violation’ determination on a single dispositive issue.”); *Boston Sci. Scimed, Inc. v. Cook Grp., Inc.*, 809 F. App’x 984, 990 (Fed. Cir. 2020) (recognizing that “[t]he Board has the discretion to decline to decide additional instituted grounds once the petitioner has prevailed on all its challenged claims”).

Petitioner has not shown that the challenged claims 11 and 29 are unpatentable over Linhult, Abrahmsén, and Hober. We note that each of Linhult, Abrahmsén, and Hober is silent with respect to Fab binding to a mutant SPA domain. *See above* II.E.5.b. Patent Owner asserts, and we agree, that there is no reasonable expectation that a G29A SPA domain mutant would bind Fab. Specifically, Patent Owner’s cited references show that a G29A mutation in domain B results in a domain Z matrix composition that *does not* bind Fab. *See* Ex. 2029, 6 (Fig. 3 (*compare* Panel B-domain B, *with* Panel B-domain Z); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”). For the reason discussed above (II.E.5.b), Petitioner has not shown by a preponderance of the evidence of record that a G29A SPA domain mutant would bind a Fab fragment so that a process of using the G29A SPA domain ligand would reasonably result in the purification of the Fab target. That deficiency in Petitioner’s showing persists whether the grounds are based on the combination of Linhult, Abrahmsen, and Hober (discussed above) or the related combinations of Linhult combined with Abrahmsen or Hober or Abrahmsen combined with Hober.

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2. *The '045 IPR Grounds Based on Various Combinations of Berg¹³, Linhult, Abrahmsén, and Hober*

Petitioner asserts that claims 1–14, 16–18, 20–32, and 34–36 are unpatentable as obvious over Berg¹⁴ and Linhult ('045 IPR Pet. 21–39); claims 4–8, 10, 19, 22–26, 28, and 37 are unpatentable as obvious over Berg, Linhult, and Hober (*id.* at 39–46); that claims 1–3, 9, 10, 12–14, 16–18, 20, 21, 27, 28, 30–32, and 34–36 are unpatentable as obvious over Berg and Abrahmsén (*id.* at 47–54); and that claims 4–8, 10, 11, 19, 22, 26, 28, 29, and 37 are unpatentable as obvious over Berg, Abrahmsén, and Hober (*id.* at 54–57) under 35 U.S.C. §103(a).

Berg relates to a chromatography matrix to which antibody-binding protein ligands are immobilized. Ex. 1018, Abstract. Petitioner relies on a single paragraph in Berg, paragraph 29, for teaching antibody binding ligands including SPA domain C. *See* '045 IPR Pet. 24–25, 48. The remainder of the Berg reference is directed to the structure of the chromatography matrix. *See generally* Ex. 1018. A review of Berg shows that SPA is mentioned at three locations in the reference. *See* Ex. 1018 ¶¶ 28, 29, and claim 12. Paragraph 29 of Berg suggests using ligands made up of one or more domains A, B, C, D, and E, and preferably domain B and/or domain C. Ex. 1018 ¶ 29. Just like Linhult, Abrahmsén, and Hober, Berg

¹³ Berg et al., US 2006/0134805 A1, published June 22, 2006. Ex. 1018.

¹⁴ We recognize that there is a dispute between the parties whether Berg qualifies as a 35 U.S.C. §102(a) date reference or a §102(b) date reference. *See* Pet; PO Resp., Reply, and Sur-reply. Because we do not need to reach these additional asserted grounds based on Berg beyond addressing whether Berg teaches Fab binding in the context of a mutant SPA domain ligand to establish that Berg does not address the deficiency of the Linhult, Abrahmsén, and Hober combination, we, therefore, do not need to address the prior art status of Berg.

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also does not say anything about the ability of a mutant SPA domain ligand, including a domain C ligand, to bind Fab.

Patent Owner, however, cites prior art references to establish that at the time the invention was made there was no expectation that a G29A SPA domain mutant would bind Fab. *See above* II.E.5.b; *see* '045 IPR PO Resp. 43, 54, 55, 58. Specifically, Patent Owner's cited references showing that a G29A mutation in domain B results in a domain Z matrix composition that does not bind Fab. *See id.*; Ex. 2029, 6 (Fig. 3 (compare Panel B-domain B, with Panel B-domain Z); Ex. 2013, 3 (“[t]he site responsible for Fab binding is structurally separate from the domain surface that mediates Fcγ binding”).

Berg, therefore, does not address Fab binding in the context of a mutant SPA domain ligand, specifically domain C, nor does Berg explain why one of ordinary skill in the art would have reasonably expected domain C to retain the ability to bind Fab when other SPA domain mutants do not retain this feature. Because Berg does not address the deficiency of Linhult, Abrahmsén, and Hober as identified by Patent Owner and discussed above (II.E.5.b), any combination of Berg in conjunction with Linhult, Abrahmsén, and/or Hober would not address the missing limitation of claims 11 and 29. Therefore, we do not reach these additional asserted grounds based on Berg beyond addressing whether Berg addresses this missing limitation. *See Beloit Corp.*, 742 F.2d at 1423.

III. CONCLUSION¹⁵

For the foregoing reasons, we determine that Petitioner has demonstrated by a preponderance of the evidence that claims 1–10, 12–14,

¹⁵ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this

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16–28, 30–32, and 34–37 of the '007 patent are unpatentable, and that claims 11 and 29 have not been shown unpatentable on the bases set forth in the following table.

In summary:

IPR2022-00042

Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–11, 20–29	103(a)	Linhult, Abrahmsén ¹⁶		11, 29
1–14, 16–32, 34–37	103(a)	Linhult, Hober ¹⁷		11, 29
1–14, 16–32, 34–37	103(a)	Linhult, Abrahmsén, Hober	1–10, 12–14, 16–28, 30–32, 34–37	11, 29
1–14, 16–32, 34–37	103(a)	Abrahmsén, Hober ¹⁸		11, 29

decision, we draw Patent Owner's attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. See 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. See 37 C.F.R. § 42.8(a)(3), (b)(2).

¹⁶ As explained above (II.F.1), we do not reach claims 1–10 and 20–28 in this '042 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these claims.

¹⁷ As explained above (II.F.1), we do not reach claims 1–10, 12–14, 16–28, 30–32, and 34–37 in this '042 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

¹⁸ As explained above (II.F.1), we do not reach claims 1–10, 12–14, 16–28, 30–32, and 34–37 in this '042 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged *claims*.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
Overall Outcome			1–10, 12–14, 16–28, 30–32, 34–37	11, 29

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
1–14, 16– 18, 20–32, 34–36	103(a)	Berg, Linhult ¹⁹		11, 29
4–8, 10, 19, 22–26, 28, 37	103(a)	Berg, Linhult, Hober ²⁰		
1–3, 9, 10, 12–14, 16– 18, 20, 21, 27, 28, 30– 32, 34–36	103(a)	Berg, Abrahmsén ²¹		

¹⁹ As explained above (II.F.2), we do not reach claims 1–10, 12–14, 16–18, 20–28, 30–32, and 34–36 in this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of all the challenged claims.

²⁰ As explained above (II.F.2), we do not reach this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

²¹ As explained above (II.F.2), we do not reach this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

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Claim(s)	35 U.S.C. §	Reference(s)/Basis	Claim(s) Shown Unpatentable	Claim(s) Not shown Unpatentable
4–8, 10, 11, 19, 22, 26, 28, 29, 37	103(a)	Berg, Abrahmsén, Hober ²²		11, 29
Overall Outcome			1–10, 12–14, 16–28, 30–32, 34–37	11, 29

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the preponderance of the evidence of record has shown that claims 1–10, 12–14, 16–28, 30–32, and 34–37 of the '007 patent are found unpatentable;

ORDERED that the preponderance of the evidence of record has not shown that claims 11 and 29 of the '007 patent are found unpatentable; and

FURTHER ORDERED because this is a final written decision, the parties to this proceeding seeking judicial review of our Decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

²² As explained above (II.F.2), we do not reach claims 4–8, 10, 19, 22, 26, 28, 37 in this '045 IPR ground because the challenge based on the Linhult, Abrahmsén, and Hober ground is dispositive of these challenged claims.

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IPR2022-00045
Patent 10,875,007 B2

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US010213765B2

(12) **United States Patent**
Hall et al.(10) **Patent No.: US 10,213,765 B2**(45) **Date of Patent: *Feb. 26, 2019**(54) **CHROMATOGRAPHY LIGAND
COMPRISING DOMAIN C FROM
STAPHYLOCOCCUS AUREUS PROTEIN A
FOR ANTIBODY ISOLATION***B01J 20/28019* (2013.01); *B01J 20/3212*
(2013.01); *B01J 20/3219* (2013.01); *B01J*
20/3274 (2013.01); *B01J 20/3293* (2013.01);
C07K 1/22 (2013.01); *C07K 14/31* (2013.01);
C07K 16/00 (2013.01); *C07K 17/00* (2013.01);
C07K 17/10 (2013.01); *B01J 2220/52*
(2013.01); *B01J 2220/54* (2013.01); *C07K*
2317/55 (2013.01)(71) Applicant: **GE Healthcare BioProcess R&D AB,**
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Rodrigo, Uppsala (SE); **Jinyu Zou,**
Uppsala (SE); **Per-Mikael Aberg,**
Uppsala (SE)(58) **Field of Classification Search**

None

See application file for complete search history.

(73) Assignee: **GE Healthcare Bioprocess R&D,**
Uppsala (SE)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.This patent is subject to a terminal dis-
claimer.(21) Appl. No.: **15/603,285**(22) Filed: **May 23, 2017**(65) **Prior Publication Data**

US 2017/0259244 A1 Sep. 14, 2017

Related U.S. Application Data(60) Continuation of application No. 15/063,471, filed on
Mar. 7, 2016, now Pat. No. 9,663,559, which is a
division of application No. 14/164,519, filed on Jan.
27, 2014, now Pat. No. 9,290,549, which is a
continuation of application No. 13/559,663, filed on
Jul. 27, 2012, now Pat. No. 8,772,447, which is a
division of application No. 12/443,001, filed as
application No. PCT/SE2007/000862 on Sep. 27,
2007, now Pat. No. 8,329,860.(30) **Foreign Application Priority Data**

Sep. 29, 2006 (SE) 0602061

(51) **Int. Cl.***C07K 1/22* (2006.01)
B01D 15/38 (2006.01)
B01J 20/24 (2006.01)
B01J 20/286 (2006.01)
B01J 20/32 (2006.01)
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C07K 16/00 (2006.01)
C07K 17/00 (2006.01)
B01J 20/28 (2006.01)
B01J 20/285 (2006.01)
B01J 20/289 (2006.01)
C07K 17/10 (2006.01)(52) **U.S. Cl.**CPC *B01J 20/24* (2013.01); *B01D 15/3809*
(2013.01); *B01J 20/285* (2013.01); *B01J*
20/286 (2013.01); *B01J 20/289* (2013.01);(56) **References Cited**

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Primary Examiner — Brian Gangle(74) *Attorney, Agent, or Firm* — Arent Fox, LLP(57) **ABSTRACT**The present invention relates to a chromatography ligand,
which comprises Domain C from *Staphylococcus* protein A
(SpA), or a functional fragment or variant thereof. The
chromatography ligand presents an advantageous capability
of withstanding harsh cleaning in place (CIP) conditions,
and is capable of binding Fab fragments of antibodies. The
ligand may be provided with a terminal coupling group, such
as arginine or cysteine, to facilitate its coupling to an
insoluble carrier such as beads or a membrane. The inven-
tion also relates to a process of using the ligand in isolation
of antibodies, and to a purification protocol which may
include washing steps and/or regeneration with alkali.**26 Claims, 2 Drawing Sheets****Specification includes a Sequence Listing.**

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Sheet 1 of 2

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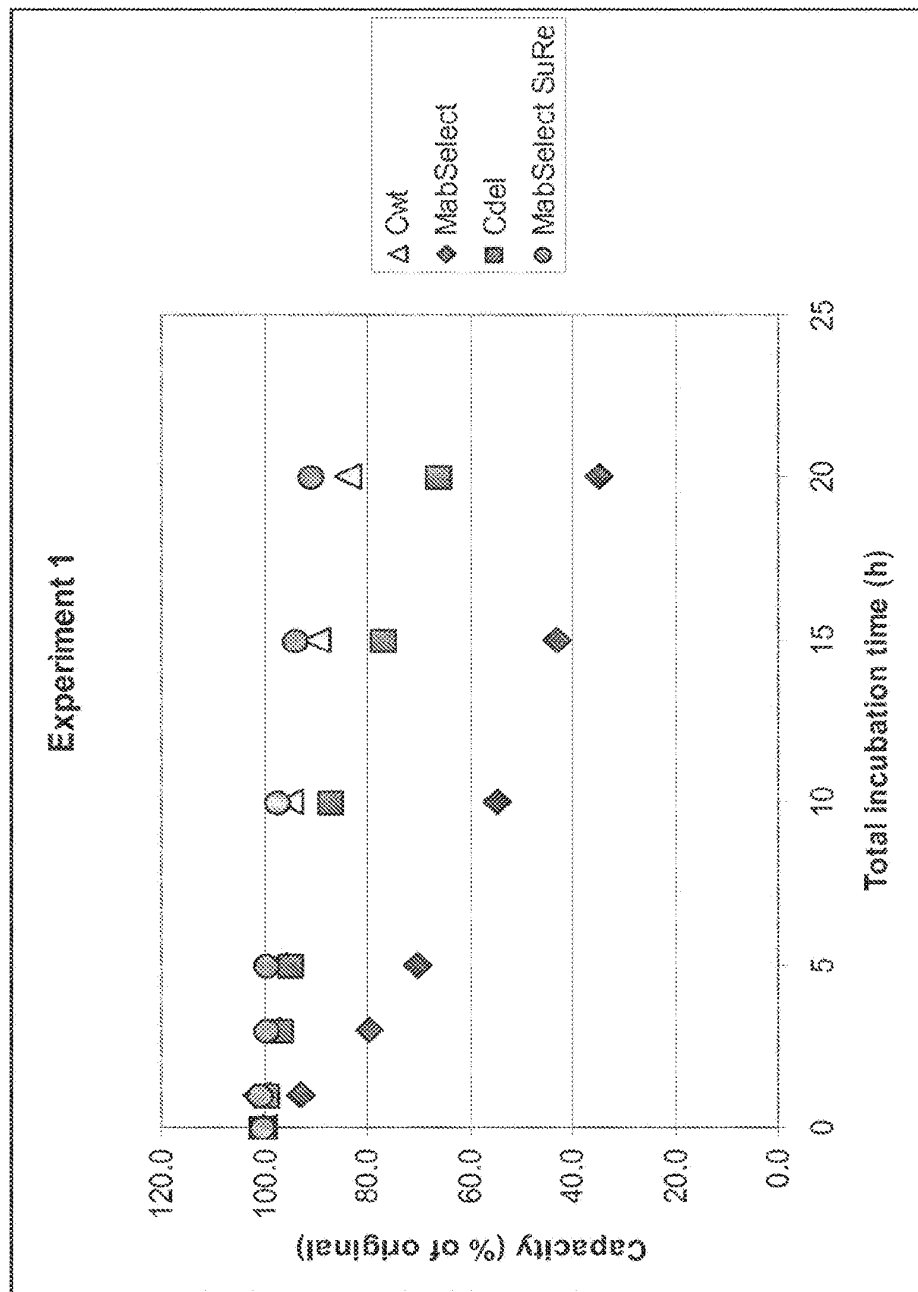


Figure 1

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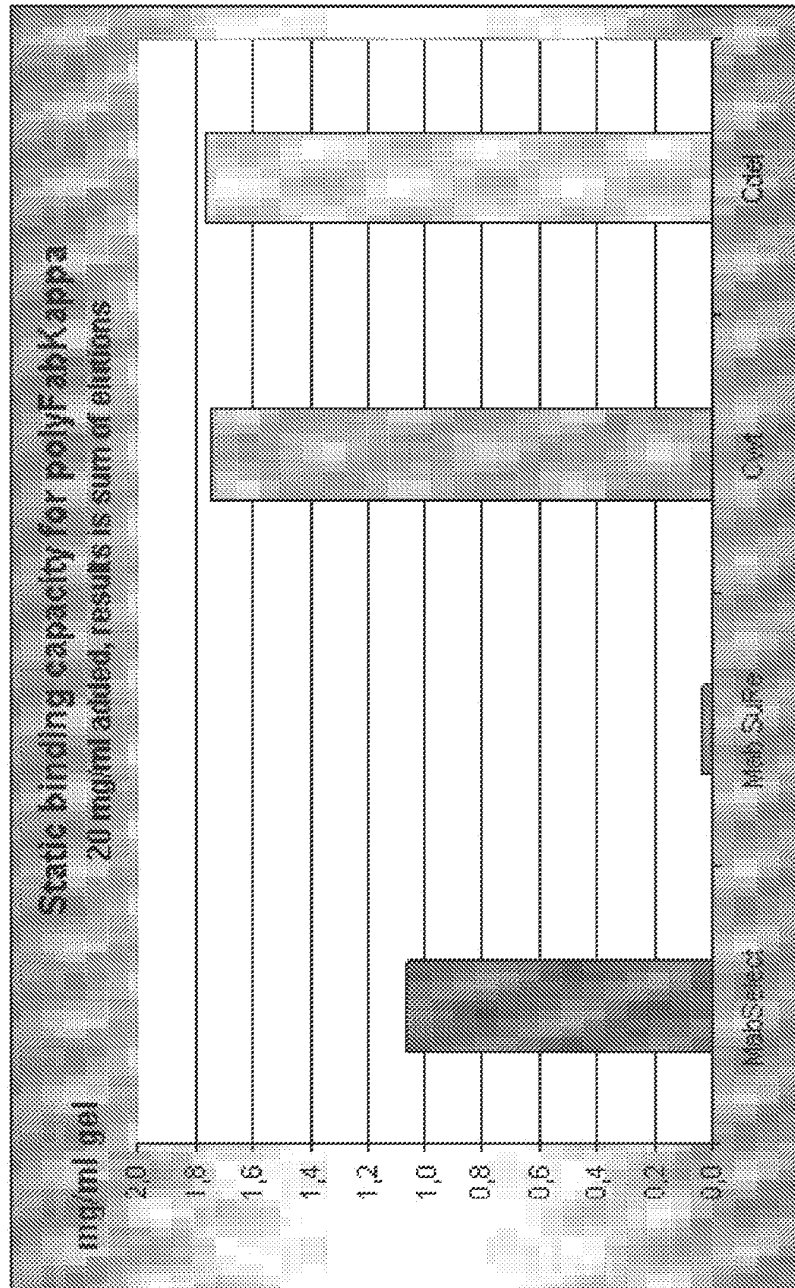


Figure 2

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**CHROMATOGRAPHY LIGAND
COMPRISING DOMAIN C FROM
STAPHYLOCOCCUS AUREUS PROTEIN A
FOR ANTIBODY ISOLATION**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 15/063,471, filed on Mar. 7, 2016, which will issue as U.S. Pat. No. 9,663,559 on May 30, 2017, which is a division of U.S. patent application Ser. No. 14/164,519, filed Jan. 27, 2014, now U.S. Pat. No. 9,290,549, which is a continuation of U.S. patent application Ser. No. 13/559,663, filed Jul. 27, 2012, now U.S. Pat. No. 8,772,447, which is a division of U.S. patent application Ser. No. 12/443,011 filed on Mar. 26, 2009, now U.S. Pat. No. 8,329,860, which is a filing under 35 U.S.C. § 371 and claims priority to international patent application number PCT/SE2007/000862 filed Sep. 27, 2007, published on Apr. 3, 2008, as WO 2008/039141, which claims priority to patent application number 0602061-4 filed in Sweden on Sep. 29, 2006.

STATEMENT REGARDING SEQUENCE
LISTING

The Sequence Listing associated with this application is part of the description and is provided in text the form of an Annex C/ST.25 text file in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 220662-19 PU06101 CON DIV1CON_SequenceListing.txt. The text file is 2 kb, was created on May 23, 2017, and is being submitted herewith electronically via EFS-Web.

FIELD OF THE INVENTION

The present invention relates to the field of chromatography, and more specifically to a novel affinity ligand which is suitable for use in antibody isolation. Thus, the invention encompasses affinity ligands as such, a chromatography matrix comprising ligands according to the invention, and a process of antibody isolation, wherein the ligand according to the invention is used.

BACKGROUND OF THE INVENTION

The term chromatography embraces a family of closely related separation methods based on the contacting of two mutually immiscible phases, wherein one phase is stationary and the other phase is mobile. One area wherein chromatography is of great interest is in the biotechnological field, such as for large-scale economic production of drugs and diagnostics. Generally, proteins are produced by cell culture, either intracellularly or by secretion into the surrounding medium. Since the cell lines used are living organisms, they must be fed with a complex growth medium containing sugars, amino acids, growth factors, etc. Separation of the desired protein from the mixture of compounds fed to the cells and from other cellular components to a sufficient purity, e.g. for use as a human therapeutic, poses a formidable challenge.

In such separation, in a first step, cells and/or cell debris is usually removed by filtration. Once a clarified solution containing the protein of interest has been obtained, its separation from the other components of the solution is often performed using a combination of different chromatography

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steps, often based on different separation principles. Thus, such steps separate proteins from mixtures on the basis of charge, degree of hydrophobicity, affinity properties, size etc. Several different chromatography matrices, such as matrices for ion exchange, hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC), affinity chromatography and immobilized metal affinity chromatography (IMAC), are available for each of these techniques, allowing tailoring of the purification scheme to the particular protein involved. An illustrative protein, which is of steadily growing interest in the medical field, is immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG).

As in all process technology, an important aim is to keep the production costs low. Consequently, improved chromatographic techniques are frequently presented, and the matrices are when possible reused. However, since each use of a chromatography matrix will leave certain traces of the operation just performed, many different cleaning protocols are available for cleaning and/or restoring the matrix into its original form. Commonly known materials that need to be removed are e.g. non-eluted proteins and protein aggregates as well as potentially hazardous materials, such as virus, endotoxins etc., which may originate from the cell culture. The most commonly used cleaning is a simple wash with buffer. For a more efficient cleaning of the matrix, treatments with acid and/or base are frequently used. In order to even more efficiently restore the matrix, an alkaline protocol known as Cleaning In Place (CIP) is commonly used. The standard CIP involves treatment of the matrix with 1M NaOH, pH 14. Such harsh treatment will efficiently remove undesired fouling of the above-discussed kind, but may in addition impair some chromatography matrix materials. For example, many affinity matrices, wherein the ligands are proteins or protein-based, cannot withstand standard CIP, at least not while maintaining their original properties. It is known that structural modification, such as deamidation and cleavage of the peptide backbone, of asparagine and glutamine residues in alkaline conditions is the main reason for loss of activity upon treatment of protein in alkaline solutions, and that asparagine is the most sensitive of the two. It is also known that the deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times in proteins have been associated with the sequences -asparagine-glycine- and -asparagine-serine. See e.g. Gülich, Linhult, Nygren, Uhlen and Hober (2000) Journal of Biotechnology 80, 169-178. Stability towards alkaline conditions can be engineered into a protein ligand.

Despite the documented alkaline sensitivity, protein A is widely used as a ligand in affinity chromatography matrices due to its ability to bind IgG without significantly affecting the affinity of immunoglobulin for antigen. As is well known, Protein A is a constituent of the cell wall of the bacterium *Staphylococcus aureus*. Such *Staphylococcus* protein, known as SpA, is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind antibodies at the Fc region, and a C-terminal region (or "X" region) that does not bind any antibodies. Jansson et al (Jansson, Uhlen and Nygren (1998) FEMS Immunology and Medical Microbiology 20, 69-78: "All individual domains of staphylococcal protein A show Fab binding") have later shown that all the individual SpA domains also bind certain antibodies at the Fab region.

U.S. Pat. No. 5,151,350 (Repligen) relates to cloning and expression of the gene coding for a protein A and protein A-like material. The cloning of this gene with its nucleotide sequence characterization enabled in 1982 for the first time

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to obtain quantities of a protein A-like material and nucleotide sequence for cloning in various host-vector systems.

Since the production of protein A in a recombinant system was accomplished, further genetic manipulations thereof have been suggested. For example, U.S. Pat. No. 5,260,373 (Repligen) describes genetic manipulation of recombinant protein A in order to facilitate the attachment thereof to a support, and more specifically to the coupling thereof via arginine. Further, U.S. Pat. No. 6,399,750 (Pharmacia Biotech AB) describes another recombinant protein A ligand, which has been coupled to a support via cysteine.

However, in order to maintain selectivity and binding capacity, Protein A chromatography matrices of the above-discussed kind need to be cleaned under milder conditions than conventional CIP. In this context, it is understood that the cleaning is closely related to the lifetime of the chromatography matrix. For example, a sensitive matrix may be cleaned with standard CIP, if a reduced performance is acceptable. Thus, efforts have been made to provide chromatography matrices which present the outstanding properties, such as selectivity, of protein A, but which are more resistant to alkaline conditions used for CIP.

Thus, U.S. Pat. No. 6,831,161 (Uhlén et al) relates to methods of affinity separation using immobilized proteinaceous affinity ligands, wherein one or more asparagine (Asn) residues have been modified to increase alkaline stability. This patent also describes methods of making a stabilized combinatorial protein by modification of Asn residues within a protein molecule to increase stability of the protein in alkaline conditions, and randomization of a protein molecule to modify its binding characteristics, and combinatorial proteins wherein in a step separate from the randomization step, the stability of the protein in alkaline conditions has been increased by modifying one or more of its Asn residues.

Further, WO 03/080655 (Amersham Biosciences) relates to an immunoglobulin-binding protein, wherein at least one asparagine residue has been mutated to an amino acid other than glutamine or aspartic acid. According to this patent application, such more specific mutation confers an increased chemical stability at pH-values of up to about 13-14 compared to the parental molecule. The mutated protein can for example be derived from a protein capable of binding to other regions of the immunoglobulin molecule than the complementarily determining regions (CDR), such as protein A, and preferably from the B-domain of Staphylococcal protein A. The invention also relates to a matrix for affinity separation, which comprises the described mutated immunoglobulin-binding proteins as ligands.

Despite the above-described development towards more alkaline-stable protein A-based chromatography ligands, there is still a need in this field of improved ligands and chromatography matrices for highly specific isolation of antibodies, and of alternative wild type ligand constructions that allow easier manufacture.

One example of such an improved chromatography matrix is described in US 2006/0134805 (Berg et al), which relates to a separation matrix comprised of porous particles to which antibody-binding protein ligands have been immobilised. More specifically, the disclosed chromatography matrix has been optimised in terms of ligand density; gel phase distribution coefficient (K_{av}); and particle size to provide a matrix especially suitable for high capacity purification of antibodies. The ligands of the disclosed matrix may comprise antibody-binding protein such as Protein A, Protein G and/or Protein L.

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SUMMARY OF THE INVENTION

One aspect of the present invention is to provide a novel chromatography ligand, which is capable of withstanding repeated cleaning-in-place cycles. This may be achieved by an affinity ligand which is based on domain C from SpA Domain C, as defined in the appended claims.

Another aspect of the present invention is to provide an economical process of purifying immunoglobulins. This may be achieved by a process which uses an affinity chromatography ligand capable of withstanding repeated cleaning-in-place cycles.

Further aspects and advantages of the invention will appear from the detailed disclosure that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows results of testing the alkaline-stability of the ligand according to the invention as compared to other protein-based ligands. The X axis shows the incubation time in hours; while the Y axis shows the capacity that remains after X hours in 0.5M NaOH, as described in Example 1. More specifically, the Protein A-containing product MAB-SELECT™ (◆); the more recent Protein A product MAB-SELECT SURE™, marketed as more alkaline-stable (X); Domain C from SpA as defined by SEQ ID NO 1 (Δ); and finally a deleted embodiment of Domain C from SpA as defined by SEQ ID NO 2 (■). As appears from FIG. 1, the Domain C ligand according to the invention shows an alkaline-stability well comparable to the alkaline-stable product MABSELECT SURE™.

FIG. 2 shows the results of testing the Fab-binding properties of the ligand according to the invention, as compared to other protein-based ligands. As appears from this figure, a chromatography ligand comprising Domain C from SpA (Cwt and Cdel) present a much higher levels of Fab-binding than the other tested ligands.

DEFINITIONS

The term Domain C or “functional fragments or variants thereof” encompasses fragments or variants of SpA Domain C, which have the property of binding to IgG at the Fc region. The terms “antibody” and “immunoglobulin” are used interchangeably herein, and are understood to include also fusion proteins comprising antibodies and fragments of antibodies.

The term an “Fc-binding protein” means a protein capable of binding to the crystallisable part (Fc) of an antibody and includes e.g. Protein A and Protein G, or any fragment or fusion protein thereof that has maintained said binding property.

The term “Fab fragment” refers to the variable part of an antibody; hence a “Fab-binding ligand” is capable of binding to either full antibodies via Fab-binding; or to antibody fragments which includes the variable parts also known as Fab fragments.

The term “chromatography” is used herein for any kind of separation which utilises the principles of chromatography, and hence includes batch as well as HPLC methods.

The term “affinity chromatography” is used herein for the specific mode of chromatography where the ligand interacts with target via biological affinity in a “lock-key” fashion. Examples of useful interactions in affinity chromatography are e.g. enzyme-substrate interaction, biotin-avidin interaction, antibody-antigen interaction etc.

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The term “protein-based” ligands means herein ligands which comprise a peptide or protein; or a part of a peptide or a part of a protein.

The term “isolation” of an antibody is used herein as embracing purification of a specific product antibody from a mixture comprising other proteins, such as other antibodies, and other components; as well as the separation of an antibody from a product liquid, i.e. to remove an undesired antibody.

DETAILED DESCRIPTION OF THE INVENTION

Thus, the present invention relates to a novel chromatography ligand. The chromatography ligand according to the invention, which is protein-based and of the kind known as affinity ligand, comprises all or parts of Domain C from *Staphylococcus* protein A (SpA). In a first aspect, the present invention relates to a chromatography ligand, which ligand comprises one or more Domain C units from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof. In one embodiment, the present chromatography ligand is substantially alkaline-stable. In this context, the term “substantially alkaline-stable” is understood to mean that the ligand is capable of withstanding repeated cleaning-in-place cycles using alkaline wash liquid without losing its binding capacity.

In a specific embodiment, the present invention is a chromatography ligand, which comprises Domain C from *Staphylococcus* protein A (SpA), but none of the other domains of SpA.

In an alternative aspect, the present invention relates to a chromatography ligand, which ligand comprises one or more Domain C units from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof, which chromatography ligand is capable of binding to the Fab part of antibodies, as discussed in more detail below.

As discussed above, Jansson et al have already shown that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A. The present inventors have confirmed that the immunoglobulin binding properties of Domain C are fully satisfactory for the use thereof as a chromatography ligand. As also discussed above, Gülich and others had shown that asparagine and glutamine residues in alkaline conditions is the main reason for loss of protein A activity upon treatment in alkaline solutions, and that asparagine is the most sensitive of the two. Consequently, the Domain C ligand, which contains as many as six asparagine residues, was not be expected to present any substantial alkaline-stability as compared to protein A.

However, as shown in the experimental part below, and in FIG. 1, the present inventors have quite surprisingly shown that the SpA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product (MABSELECT™, GE Healthcare, Uppsala, Sweden) by incubation in alkaline conditions for durations as long as 20 hours. In fact, the Domain C ligand presents values of alkaline-stability which are similar to those of the product marketed as alkaline-stable (MABSELECT SURE™, GE Healthcare, Uppsala, Sweden), wherein asparagine residues have been mutated to other amino acids.

In addition to this, as discussed above, it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences -asparagine-glycine- and -asparagine-serine. Quite surprisingly, the Domain C ligand of the invention

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presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29, using the conventional numbering of the residues of Domain C.

In one embodiment, the ligand according to the invention is able to resist at least 10 hours in 0.5 M NaOH, without deviating more than about 10%, and preferably no more than 5%, from its original immunoglobulin binding capacity. Thus, after 5 hours, it will not deviate more than 10%, preferably 5% from its original binding capacity. In other words, one embodiment of the present invention is a ligand as described above, which after 5 hours incubation in 0.5M NaOH has retained at least 95% of its original binding capacity.

In an advantageous embodiment, the ligand according to the invention is able to resist at least 15 hours in 0.5 M NaOH without losing more than about 20%, and preferably no more than 10%, of its original immunoglobulin binding capacity. In a more advantageous embodiment, the ligand according to the invention is able to resist at least 20 hours in 0.5 M NaOH without losing more than about 30%, and preferably no more than 15%, of its original immunoglobulin binding capacity. In other words, one embodiment of the present invention is a ligand as described above, which after 15 hours incubation in 0.5M NaOH has retained at least 80%, advantageously at least 90% of its original binding capacity.

The skilled person in this field can easily test alkaline-stability by incubating a candidate ligand with sodium hydroxide e.g. as described in the experimental part, and subsequent testing of the binding capacity by routine chromatography experiments.

As easily realised by the skilled person in this field, a chromatography ligand according to the invention may consist of the wild type SpA Domain C amino acid sequence, as shown in SEQ ID NO 1, herein denoted Cwt. In an alternative embodiment, the chromatography ligand according to the invention consists of a functional fragment of SpA Domain C, such as the one shown in SEQ ID NO 2, which discloses a sequence herein denoted Cdel, wherein Asn-Lys-Phe-Asn in positions 3-6 have been deleted as compared to the wild type SpA Domain C sequence. In yet an alternative embodiment, a variant of SpA Domain C is prepared by adding one or more amino acids e.g. to either end of the wild type SpA Domain C amino acid sequence; or by mutation of the wild type SpA Domain C amino acid sequence, provided that such mutation does not substantially interfere with the herein described properties relating to immunoglobulin-binding and alkaline-stability. Thus, in a specific embodiment, the chromatography ligand according to the invention comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A. Alternatively, the chromatography ligand according to this embodiment comprises the deleted SpA Domain C, as shown in SEQ ID NO 2, which consequently comprises said mutation in position 25 (i.e. G25A). As the skilled person will recognise, such addition, mutation or deletion of amino acids as compared to the wild type sequence should preferably not substantially affect the folding pattern of the SpA Domain C ligand.

Thus, in one embodiment, the amino acid sequence of the ligand according to the present invention is the sequence defined by SEQ ID NO 1. In a specific embodiment, the ligand according to the invention comprises at least 60%, advantageously at least 80%, more advantageously at least 90% and most advantageously at least 95%, such as about 98% of the amino acids shown in SEQ ID NO 1. In a specific

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embodiment, the ligand according to the invention comprises at least 35, advantageously at least 46, more advantageously at least 52 and most advantageously at least 55, such as 57, of the amino acids shown in SEQ ID NO 1.

In an alternative embodiment, the amino acid sequence of the ligand according to the present invention is the sequence defined by SEQ ID NO 2. In a specific embodiment, the ligand according to the invention comprises at least 40%, advantageously at least 77%, more advantageously at least % and most advantageously at least 94%, such as about 98% of the amino acids shown in SEQ ID NO 2. In a specific embodiment, the ligand according to the invention comprises at least 31, advantageously at least 42, more advantageously at least 48 and most advantageously at least 51, such as 53, of the amino acids shown in SEQ NO 2.

As discussed in the section Background above, methods are readily available for coupling of protein ligands via certain amino acids, preferably amino acids that contain nitrogen and/or sulphur atoms, see e.g. U.S. Pat. No. 6,399,750 or U.S. Pat. No. 5,084,559. Thus, in one embodiment, the ligand according to the invention further comprises a terminal coupling group, said group preferably comprising one or more nitrogen and/or sulphur atoms. In an advantageous embodiment, the terminal coupling group is comprised of arginine or cysteine. In one embodiment, the coupling group is in the C terminal region.

Further, the present invention also relates to a multimeric chromatography ligand (also denoted a "multimer") comprised of at least two Domain C units, or a functional fragments or variants thereof, as defined above. In one embodiment, this multimer comprises no units originating from SpA. In a specific embodiment, the multimer comprises no other protein-based units. In another embodiment, the multimer comprises no other unit capable of any substantial interaction with a target such as an antibody or a Fab fragment, thus it comprises no other ligand unit. As the skilled person in this field will realise, making a multimer may require adding one or more peptides as linkers between the units. Thus, a multimer limited to containing only Domain C units according to the invention may in addition comprise linkers allowing construction of a multimer wherein each Domain C unit is sufficiently exposed to be able to participate in the binding of target.

In another embodiment, the multimer comprises one or more additional units, which are different from Domain C and preferably protein-based and equally alkaline-stable as Domain C. Thus, in the multimer, the ligand according to the invention may be repeated and/or combined with other units from other sources, such as other proteins. In one embodiment, the multimer is comprised of 2-8 units, such as 4-6 units. In one embodiment, one or more linker sequences are inserted between the multimer units. Such linkers may e.g. be inserted to allow the actual ligand units to maintain their folding pattern. Linkers in this context are well known, and the skilled person can easily decide on suitable amino acids and chain lengths which do not interfere with the herein discussed properties of the ligand. In a specific embodiment, the chromatography ligand according to the invention comprises no other SpA domains than Domain C.

In a second aspect, the present invention relates to a nucleic acid sequence encoding a chromatography ligand as described above. Thus, the invention encompasses all forms of the present nucleic acid sequence such as the RNA and the DNA encoding the ligand. The invention embraces a vector, such as a plasmid, which in addition to the coding sequence comprises the required signal sequences for expression of the ligand according to the invention. In one embodiment, the

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vector comprises nucleic acid encoding a multimeric ligand according to the invention, wherein the separate nucleic acids encoding each unit may have homologous or heterologous DNA sequences. This aspect also embraces an expression system comprising a nucleic acid sequence encoding a ligand according to the invention. The expression system may e.g. be a prokaryotic host cell system, e.g. *E. coli* which has been modified to express the present ligand. In an alternative embodiment, the expression system is a eukaryotic host cell system, such as a yeast.

As the skilled person in this field will appreciate, the ligand according to the invention may alternatively be produced by protein synthesis methods, wherein the ligand is obtained by an automated process adding amino acids one at a time following a predetermined sequence. In an advantageous embodiment, segments of amino acids amino acid sequences are synthesized and linked to each other to prepare the ligand according to the invention. Such synthesis and linking procedures are well known to the skilled person in this field.

In a third aspect, the present invention relates to a chromatography matrix comprised of ligands as described above coupled to an insoluble carrier. Such a carrier may be one or more particles, such as beads or irregular shapes; membranes; filters; capillaries; monoliths; and any other format commonly used in chromatography. Thus, in an advantageous embodiment of the matrix, the carrier is comprised of substantially spherical particles, also known as beads. Suitable particle sizes may be in the diameter range of 5-500 μm , such as 10-100 μm , e.g. 20-80 μm . In an alternative embodiment, the carrier is a membrane. To obtain high adsorption capacities, the carrier is preferably porous, and ligands are then coupled to the external surfaces as well as to the pore surfaces. Thus, in an advantageous embodiment of the matrix according to the invention, the carrier is porous.

The carrier may be made from an organic or inorganic material. In one embodiment, the carrier is prepared from a native polymer, such as cross-linked carbohydrate material, e.g. agarose, agar, cellulose, dextran, chitosan, konjac, carrageenan, gellan, alginate etc. The native polymer carriers are easily prepared and optionally cross-linked according to standard methods, such as inverse suspension gelation (S Hjertén: *Biochim Biophys Acta* 79(2), 393-398 (1964)). In an alternative embodiment, the carrier is prepared from a synthetic polymer or copolymer, such as cross-linked synthetic polymers, e.g. styrene or styrene derivatives, divinylbenzene, acrylamides, acrylate esters, methacrylate esters, vinyl esters, vinyl amides etc. Such synthetic polymer carriers are easily prepared and optionally cross-linked according to standard methods, see e.g. "Styrene based polymer supports developed by suspension polymerization" (R Arshady: *Chimica e L'Industria* 70(9), 70-75 (1988)). Native or synthetic polymer carriers are also available from commercial sources, such as GE Healthcare Bio-Sciences AB, Uppsala, Sweden, for example in the form of porous particles. In yet an alternative embodiment, the carrier is prepared from an inorganic polymer, such as silica. Inorganic porous and non-porous carriers are well known in this field and easily prepared according to standard methods.

In a fourth aspect, the present invention relates to a method of preparing a chromatography matrix, which method comprises providing ligands as described above; and coupling of said ligands to a carrier. In an advantageous embodiment, the coupling is carried out via a nitrogen or sulphur atom of the ligand. In brief, the ligands may be coupled to the carrier directly; or indirectly via a spacer

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element to provide an appropriate distance between the carrier surface and the ligand. Methods for immobilisation of protein ligands to porous or non-porous surfaces are well known in this field; see e.g. the above-discussed U.S. Pat. No. 6,399,750.

In a fifth aspect, the present invention relates to a process of isolating one or more target compounds, which process comprises contacting a liquid comprising said compound(s) with a chromatography matrix; allowing said compound(s) to adsorb to ligands present on the matrix, wherein said ligands consists of one or more *Staphylococcus* protein A (SpA) Domain C, and/or functional fragments or variants thereof; and, optionally, eluting said compound(s) by the passing across said matrix of a liquid that releases compound(s) from ligands. Thus, in this embodiment, the ligands comprise no other SpA-derived domain than Domain C, or a functional fragment or variant thereof. In an alternative embodiment, said ligands are multimers comprising two or more SpA Domain C units, or functional fragments or variants thereof.

In an advantageous embodiment, the ligands are the ligands described above. The target compound(s) may be any organic compound, biomolecule or other biological material, such as proteins, e.g. antibodies; peptides; cells, such as eukaryotic and prokaryotic cells; nucleic acids, such as DNA, e.g. plasmids, and RNA; virus; etc. In an advantageous embodiment, the target compound(s) is one or more monoclonal or polyclonal antibodies, such as IgA, IgD, IgE, IgG, and IgM. In one embodiment, the target compound is a fragment of an antibody, such as a Fab fragment. In yet another embodiment, the target compound is a fusion protein wherein at least one part is an antibody or an antibody fragment.

In one embodiment, the chromatography matrix is a disposable product, and elution will then not be required if the purpose of the process is to remove the target compound such as the antibody from a product liquid. This embodiment may e.g. be for the removal of an undesired antibody from a liquid, such as a medical liquid or a liquid wherein many antibodies are produced, such as milk from a recombinant animal.

In an alternative embodiment, when the adsorbed compound is the desired product, the elution step is included in the process. To obtain the most suitable conditions for adsorption, a liquid sample is combined with a suitable buffer or other liquid such as water to provide the mobile phase. The present method is advantageously run under conditions conventional for affinity chromatography, and especially for protein A chromatography, as is well known in this field.

In a sixth aspect, the present invention relates to the use Domain C of SpA, or a functional fragment or variant thereof, as alkaline-stable immunoglobulin adsorbent. In this context, "alkaline-stable" is understood to mean that the adsorbent alkaline-stability is not lower than about 10%, such as about 5%, below that of a commercial products marketed as being alkaline-stable, such as MABSELECT SURE™ (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) during the first 5 hours of incubation in 0.5M NaOH. In an advantageous embodiment, the adsorbent is a ligand as described above. As said MABSELECT SURE™ should present a minimal deterioration after such time and conditions, the antibody binding capacity of the adsorbent should not be lower than about 10%, such as about 5%, below its original binding capacity after such time and conditions. In this context, the term "original" refers to its capacity before

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any alkaline regeneration, and the comparisons are carried out as side-by-side experiments using a procedure of the herein disclosed kind.

In one embodiment, the use according to the invention comprises a process as described above, wherein the antibodies are eluted from the matrix and which is carried out at least once, such as 2-300 times, optionally with washing steps between; alkaline regeneration of the matrix; and finally repeating said process of isolating antibodies. Washing may e.g. be carried out with a suitable buffer, such as the buffer used to equilibrate the column. In an advantageous embodiment, the regeneration is carried out by incubation with 0.5 M NaOH.

The present invention also embraces a method of purifying one or more target compounds, as discussed above, which method comprises one or more chromatography steps in addition to the purification using the chromatography matrix according to the invention. The method according to this aspect may e.g. comprises first chromatography step using the present matrix; an intermediate chromatography step using either ion exchange or hydrophobic interaction chromatography (HIC); and finally a polishing step using ion exchange, HIC or reverse phase chromatography. In a specific embodiment, this process comprises a step preceding the chromatography matrix having Domain C ligands as described herein. Such a preceding step may e.g. be a conventional filtration, sedimentation, flocculation or other step to remove cell debris and other undesired components.

In an alternative embodiment, the use according to the invention is an analytical or diagnostic use, such as an immunoassay.

EXAMPLES

The present examples are provided as illustrative purposes only, and should not be construed as limiting the present invention as defined in the appended claims.

Example 1: Column Study of the Alkaline Stability of Four Protein A-Derived Ligands

In this example, the alkaline stability of four chromatography matrices, two of which were comparative and two of which were according to the invention, were tested through a series of chromatographic runs:

MABSELECT™ and MABSELECT SURE™ (both comparative products comprising protein-based ligands, GE Healthcare Bio-Sciences, Uppsala, Sweden), and Cwt (wild type Domain C from SpA, as defined in SEQ ID NO. 1), and Cdel (deleted wild type Domain C from SpA, as defined in SEQ ID NO. 2).

The IgG-binding capacity was measured initially and after incubation steps in 0.5 M NaOH. The incubation times varied from one to five hours, with an accumulated incubation time of 20 hours.

The ligands according to the invention were immobilized on agarose particles according to standard procedure and packed in columns (GE Healthcare). Two of the matrices, MABSELECT™ and MABSELECT SURE™, are commercial products manufactured by GE Healthcare marketed for the purification of monoclonal antibodies. The ligands of both products are based on the IgG binding *Staphylococcus aureus* Protein A. The MABSELECT™ ligand basically is recombinant Protein A, which consists of five homologous domains (E, D, A, B, C). By comparison, the MABSELECT SURE™ ligand consists of four domains which originate from the domain B analogue "Z", which in turn has been

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stabilized against high pH by protein engineering methods. As a result, MABSELECT SURE™ tolerates cleaning-in-place (CIP) conditions of up to 0.5 M NaOH. Both the MABSELECT™ and MABSELECT SURE™ ligands are coupled to agarose particles.

The ligands Cwt and Cdel were constructed as tetramers of identical domains with a C-terminal cysteine residue for coupling to a matrix according to standard procedure.

Materials & Methods

Target Compound

10×10 ml injection liquid, solution, GAMMANORM® 165 mg/ml (Octapharma no. 00 86 64), human normal immunoglobulin, for subcutane infusion or intramuscular injection, was used as the target compound in the chromatography experiments.

Chromatography Columns

Ligand coupling and column packing was carried out as outlined in Table 1 below:

TABLE 1

Columns used in Experiment 1					
Ligand/Matrix	Column ID	Column no.	Batch	Date	Column volume (ml)
MABSELECT SURE™	9	4	U669082	20060310	2.08
Cwt	11	2	U1555055A	20060310	2.02
MABSELECT™	1	7	U1555045A	20060310	2.12
Cdel	13	2	U1555059A	20060303	2.06

“Column ID” refers to a unique number given to each column. These numbers were included in the chromatography methods and can be found in the logbook of the result files. For example, the first column in table 1 was called “MABSELECT SURE™ U669082 Column 4 20060310 (9)”. “Column no.” is the packing number, i.e. columns packed with the same batch of matrix received different Column nos. upon packing. The column volume was estimated by measuring the bed height.

Buffers and Solutions

Buffer A: 50 mM Sodium phosphate, 0.15 M NaCl, pH 7.2

Buffer B: 50 mM Citric acid, 0.15 M NaCl, pH 2.5

Instruments and Laboratory Equipment

Chromatography system: ÄKTA EXPLORER™10 (GE Healthcare)

Column hardware: TWICORN™5/100 GL (GE Healthcare)

Vacuum degasser: CT 2003-2, 2 channel degasser, ChromTech AB

Spectrophotometer: NANODROP™ ND-1000 Spectrophotometer, NanoDrop Technologies

Centrifuge: Beckman Coulter AVANTI® J-20 XPI with JLA 8.1000 rotor

pH meter (Buffer A): Beckman Φ360 pH/Temp/mV Meter

pH meter (Buffer B): Laboratory pH Meter CG 842, SCHOTT

Helium: AGA Gas AB, 10 1 H 20577708, Instrument

Filter for buffer and sample: 75 mm Bottle Top Filter—500 ml, 0.2 μm pore size, Nalgene

Filter for 0.5 M NaOH: 75 mm Bottle Top Filter—500 ml, 0.45 μm pore size, Nalgene

Software

ÄKTA EXPLORER™ 10 was controlled by UNICORN™ 5.01 (GE Healthcare). Apart from controlling the

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system during the chromatography runs, UNICORN™ was used for method programming and evaluation of the results.

Buffer Preparation

Buffer A: Sodium dihydrogen phosphate and NaCl were dissolved in water. A pH meter was calibrated using pH 4, pH 7 and pH 10 standard buffers. pH was monitored while adding NaOH(aq) to the buffer until pH reached 7.2. The buffer was filtered and degassed with helium prior use.

Buffer B: Citric acid and NaCl were dissolved in water. A pH meter was calibrated using pH 7 and pH 2 standard buffers. pH was monitored while adding NaOH(aq) to the buffer until pH reached 2.5. The buffer was filtered and degassed with helium prior use.

Preparation of 0.5 M NaOH

NaOH(s) was dissolved in water to 0.5 M. The solution was filtered and degassed with helium prior use.

Sample Preparation

Experiment 1

30 ml Gammanorm (165 mg/ml) was diluted to 1 mg/ml with 4950 ml Buffer A. The sample was filtered through 0.2 μm into a sterile 5 liter bottle.

Three 280 nm absorbance measurements were performed on the sample using NANODROP™ spectrophotometer: 1.2573 AU, 1.2432 AU and 1.2101 AU. Mean absorbance: 1.2369 AU.

The absorbance at 280 nm was also measured on ÄKTA EXPLORER™ 10. The sample was pumped with the system pump through the system in bypass mode. A 10 mm UV cell was used and the flow rate was 0.83 ml/min. The absorbance at 280 nm was 1510 mAU. This value was used as a reference when making capacity calculations.

Method Description

Normally, a CIP cycle for MABSELECT SURE™ involves 10-15 minutes contact time of the CIP solution (usually 0.1-0.5 M NaOH). To reduce the amount of CIP cycles in this study, longer contact times were used. The columns were incubated for 1, 2 and 5 hour intervals, with a total contact time of 20 hours. This corresponds to 80 to 120 cycles with 10-15 minutes contact time.

Prior to the CIP incubations two initial capacity measurements were performed per column. After the capacity measurements the columns were incubated in 0.5 M NaOH. After each CIP incubation, one capacity measurement per column was carried out.

Schematically, the experiment was designed as follows:

Two initial capacity measurements per column.

CIP incubation, 1 hour.

One capacity measurement per column.

CIP incubation, 2 hour.

One capacity measurement per column.

CIP incubation, 2 hour.

One capacity measurement per column.

CIP incubation, 5 hour.

One capacity measurement per column.

CIP incubation, 5 hour.

One capacity measurement per column.

CIP incubation, 5 hour.

One capacity measurement per column.

System Setup:

The experiments were carried out in room temperature. However, the sample was kept on ice to avoid microbial growth. To avoid the formation of air bubbles when the cold sample was heated to room temperature, a degasser was connected between the sample and the pump. The ÄKTA EXPLORER™ 10 was equipped with a 10 mm UV cell for UV detection.

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Both buffer and sample was pumped through the system pumps. Following inlets were used:

Sample: B pump (inlet B1)

Buffer A: A pump (inlet A11)

Buffer B: A pump (inlet A12)

0.5 M NaOH: A pump (inlet A13)

Capacity Measurement, Detailed Description

Prior to a capacity measurement (consisting of one capacity measurement per column) sample was pumped in bypass mode, i.e. no column used. The purpose of this was to get “fresh” sample to each capacity measurement and to avoid loading the first volume of sample that remained in tubes and the pump in room temperature during the CIP incubations, onto the first column.

The capacity measurement method for each column consisted of following parts:

Equilibration of the column with 5 column volumes (CV) Buffer A.

Sample loading. Dynamic binding capacity is determined by loading a sample onto a column packed with the chromatography medium of interest. When the medium becomes more and more saturated with sample, the level of absorbance at 280 nm will increase due to unbound sample passing through the column. In this method, the sample was loaded onto the column until the UV_{280 nm} curve reached 15% of the 280 nm absorbance of the sample.

Wash out unbound sample. The column was washed with Buffer A until the UV_{280 nm} curve dropped below 10% of the 280 nm absorbance of the sample

Elution. Bound material was eluted with 10 CV of Buffer B.

Reequilibration with 5 CV Buffer A.

The flow rate of sample loading was 0.83 ml/min.

CIP Incubation

After each capacity measurement, except for the first of the two initial measurements, a CIP incubation was carried out. In the CIP incubation method, 3 CV of 0.5 M NaOH was pumped through each column at a flow rate of 0.83 ml/min. After this the system was set to pause. The length of the pause depended on the length of the CIP incubation time, i.e. 1 h, 2 h or 5 h. However, the time required for the system to pump NaOH through the columns was subtracted from the pause time. After a CIP incubation 3 CV of Buffer A was pumped through each column at a flow rate of 0.83 ml/min to remove the NaOH. By this procedure, all columns were exposed the same amount of time to NaOH. One more wash cycle with 3 CV Buffer A was finally carried out.

Evaluation of Chromatographic Results

Capacity was determined by measuring the volume of sample applied onto a column until the absorbance at 280 nm reached 10% of the sample absorbance. The dead volumes, i.e. the column volume, mixer and tubing from the pump to the UV cell, were subtracted from this volume. The delay volume without column was determined to 1.02 ml. The capacity values were plotted against the accumulated CIP incubation times. Relative capacity values were achieved by dividing the capacity values after the CIP cycles with the mean of the start capacity values. The relative capacity values were used for easier comparisons between the different matrices.

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TABLE 2

Results Experiment 1 -				
Capacity (mg Gammanorm/ml chromatography matrix (gcl))				
	MABSELECT SURE™	Cwt	MABSELECT™	Cdel
Start	28.38	27.43	28.98	30.86
Capacity 1				
Start	28.13	27.40	28.98	30.95
Capacity 2				
Capacity after 1 h	29.32	27.79	26.98	30.75
Capacity after 3 h	28.42	27.26	23.08	29.88
Capacity after 5 h	28.25	26.94	20.30	29.25
Capacity after 10 h	27.88	26.07	15.79	26.87
Capacity after 15 h	27.01	24.65	12.52	23.70
Capacity after 20 h	25.93	23.02	10.14	20.35

Experiment 2: Test of Fab-Binding

The Fab-binding ability of the different chromatography media was evaluated, in a 96-well filter plate assay. Liquids and chromatography media were mixed on a plate vortex instrument for 1 minute. The bottom of the wells consisted of a filter which retained liquids and the particles of the chromatography media. When subjected to centrifugation, the liquids passed through the filter and were collected in a separate 96-well collection UV-plate attached to the bottom of the filter plate. The absorbance at 280 nm of the collected liquid was measured in a plate reader and used for detection and estimation of Fab. The liquids from different steps, e.g. washing, elution, were collected in different plates and measured separately, to be able to measure the amount of Fab in individual fractions.

10% slurry was prepared of each chromatography medium.

The filter plates were loaded with 200 µl slurry/well, i.e. 20 µl medium/well.

Equilibration—5×200 µl wash in PBS

Sample incubation—100 µl of human polyclonal Fab/Kappa, IgG fragment (Bethyl) in PBS, 15 minutes

Wash—5×100 µl PBS

Elution—3×100 µl 0.1 M glycine, pH 3.0

CIP—2×10 min with 0.5 M NaOH

Analyze plates with liquids UV @ 280 nm

The results of experiment 2 are presented in FIG. 2.

The above examples illustrate specific aspects of the present invention and are not intended to limit the scope thereof in any respect and should not be so construed. Those skilled in the art having the benefit of the teachings of the present invention as set forth above, can effect numerous modifications thereto. These modifications are to be construed as being encompassed within the scope of the present invention as set forth in the appended claims.

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 2

<210> SEQ ID NO 1
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 1

Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile
 1 5 10 15

Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
 20 25 30

Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
 35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
 50 55

<210> SEQ ID NO 2
 <211> LENGTH: 54
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 2

Ala Asp Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His Leu Pro
 1 5 10 15

Asn Leu Thr Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp
 20 25 30

Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala Lys Lys Leu Asn
 35 40 45

Asp Ala Gln Ala Pro Lys
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What is claimed is:

1. A chromatography matrix comprising:
 a solid support; and
 a ligand coupled to the solid support, the ligand comprising at least two polypeptides,
 wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and
 wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.
2. The chromatography matrix of claim 1, wherein the ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.
3. The chromatography matrix of claim 1, wherein the chromatography matrix has retained at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.
4. The chromatography matrix of claim 1, wherein the ligand is capable of binding to the Fab part of an antibody.
5. The chromatography matrix of claim 1, wherein the ligand comprises a terminal coupling group comprising at least one nitrogen and/or sulfur atom(s).
6. The chromatography matrix of claim 5, wherein the terminal group comprises arginine or cysteine.
7. The chromatography matrix of claim 1, wherein the ligand is coupled to the solid support via thioether bonds.
8. The chromatography matrix of claim 1, wherein the ligand further comprises one or more other alkaline-stable protein-based units.
9. The chromatography matrix of claim 1, wherein the solid support is a polysaccharide.
10. The chromatography matrix of claim 1, wherein the solid support is comprised of substantially spherical particles.
11. The chromatography matrix of claim 1, wherein the solid support is porous.
12. The chromatography matrix of claim 1, wherein the ligand comprises an amino acid sequence that comprises 2-8 of the polypeptides.
13. The chromatography matrix of claim 1, wherein the solid support comprises two or more ligands.
14. A chromatography matrix comprising:
 a solid support; and
 a ligand coupled to the solid support, the ligand comprising at least two polypeptides,
 wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids in alignment with SEQ ID NO. 1, and
 wherein each polypeptide has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1.
15. The chromatography matrix of claim 14, wherein the ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.
16. The chromatography matrix of claim 14, wherein the chromatography matrix has retained at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.

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17. The chromatography matrix of claim 14, wherein the ligand is capable of binding to the Fab part of an antibody.

18. The chromatography matrix of claim 14, wherein the ligand comprises a terminal coupling group comprising at least one nitrogen and/or sulfur atom(s). 5

19. The chromatography matrix of claim 18, wherein the terminal group comprises arginine or cysteine.

20. The chromatography matrix of claim 14, wherein the ligand is coupled to the solid support via thioether bonds.

21. The chromatography matrix of claim 14, wherein the ligand further comprises one or more other alkaline-stable protein-based units. 10

22. The chromatography matrix of claim 14, wherein the solid support is a polysaccharide.

23. The chromatography matrix of claim 14, wherein the solid support is comprised of substantially spherical particles. 15

24. The chromatography matrix of claim 14, wherein the solid support is porous.

25. The chromatography matrix of claim 14, wherein the ligand comprises an amino acid sequence that comprises 2-8 of the polypeptides. 20

26. The chromatography matrix of claim 14, wherein the solid support comprises two or more ligands.

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(12) **United States Patent**
Hall et al.(10) **Patent No.:** **US 10,343,142 B2**(45) **Date of Patent:** **Jul. 9, 2019**(54) **CHROMATOGRAPHY LIGAND
COMPRISING DOMAIN C FROM
STAPHYLOCOCCUS AUREUS PROTEIN A
FOR ANTIBODY ISOLATION***B01J 20/28019* (2013.01); *B01J 20/3212*
(2013.01); *B01J 20/3219* (2013.01); *B01J*
20/3274 (2013.01); *B01J 20/3293* (2013.01);
C07K 1/22 (2013.01); *C07K 14/31* (2013.01);
C07K 16/00 (2013.01); *C07K 17/00* (2013.01);
C07K 17/10 (2013.01); *B01J 2220/52*
(2013.01); *B01J 2220/54* (2013.01); *C07K*
2317/55 (2013.01)(71) Applicant: **GE Healthcare BioProcess R&D AB,**
Uppsala (SE)(72) Inventors: **Martin Hall,** Uppsala (SE); **Sture**
Larsson, Uppsala (SE); **Andreas**
Muranyi, Uppsala (SE); **Gustav**
Rodrigo, Uppsala (SE); **Jinyu Zou,**
Uppsala (SE); **Per-Mikael Aberg,**
Uppsala (SE)(58) **Field of Classification Search**

None

See application file for complete search history.

(73) Assignee: **GE HEALTHCARE BIOPROCESS
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continuation of application No. 15/063,471, filed on
Mar. 7, 2016, now Pat. No. 9,663,559, which is a
division of application No. 14/164,519, filed on Jan.
27, 2014, now Pat. No. 9,290,549, which is a
continuation of application No. 13/559,663, filed on
Jul. 27, 2012, now Pat. No. 8,772,447, which is a
division of application No. 12/443,011, filed as
application No. PCT/SE2007/000862 on Sep. 27,
2007, now Pat. No. 8,329,860.(30) **Foreign Application Priority Data**

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C07K 17/10 (2006.01)(52) **U.S. Cl.**CPC *B01J 20/24* (2013.01); *B01D 15/3809*
(2013.01); *B01J 20/285* (2013.01); *B01J*
20/286 (2013.01); *B01J 20/289* (2013.01);(56) **References Cited**

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Primary Examiner — Brian Gangle(74) *Attorney, Agent, or Firm* — Arent Fox, LLP(57) **ABSTRACT**The present invention relates to a chromatography ligand,
which comprises Domain C from *Staphylococcus* protein A
(SpA), or a functional fragment or variant thereof. The
chromatography ligand presents an advantageous capability
of withstanding harsh cleaning in place (CIP) conditions,
and is capable of binding Fab fragments of antibodies. The
ligand may be provided with a terminal coupling group, such
as arginine or cysteine, to facilitate its coupling to an
insoluble carrier such as beads or a membrane. The inven-
tion also relates process of using the ligand in isolation of
antibodies, and to a purification protocol which may include
washing steps and/or regeneration with alkali.**30 Claims, 2 Drawing Sheets****Specification includes a Sequence Listing.**

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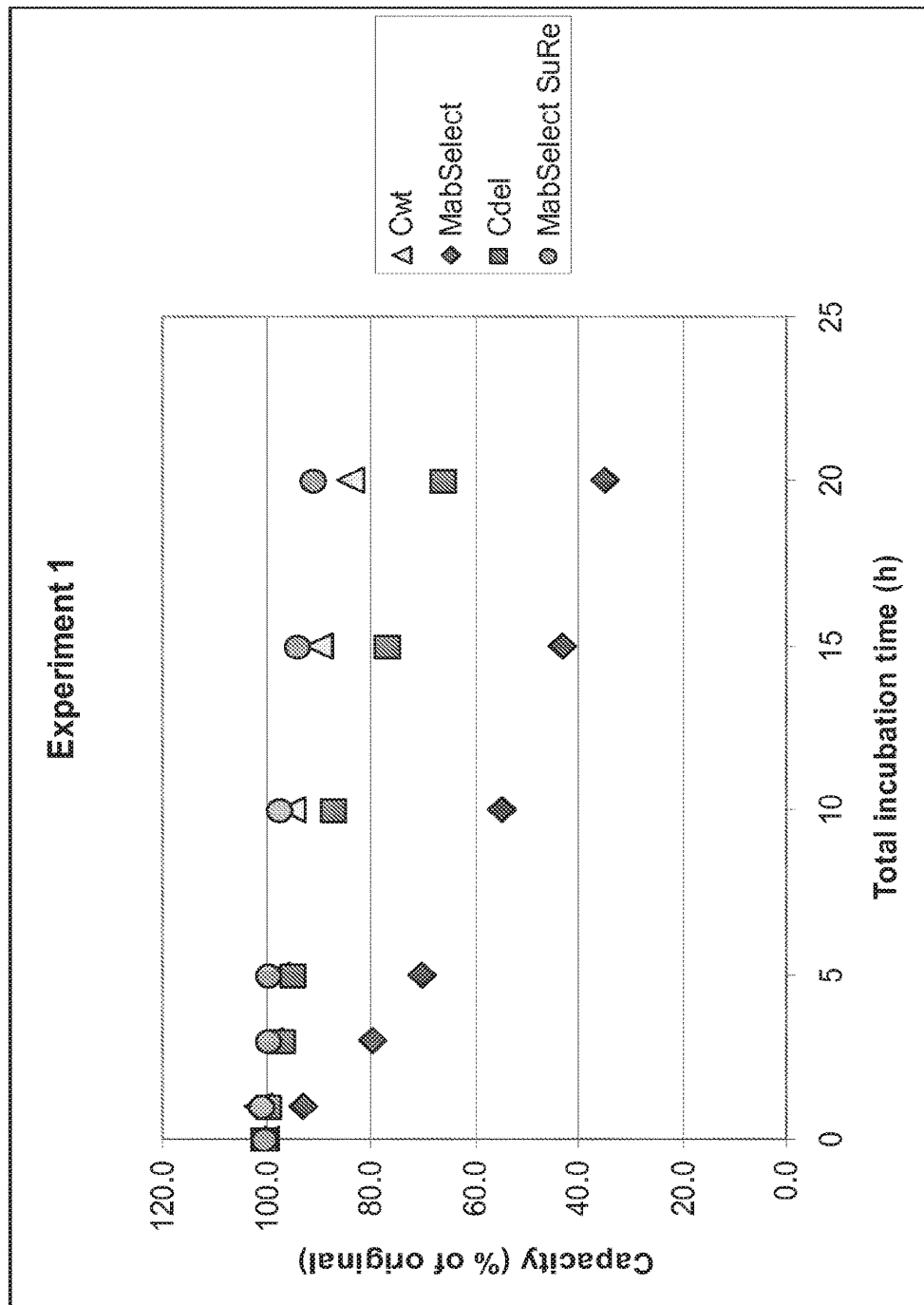


Figure 1

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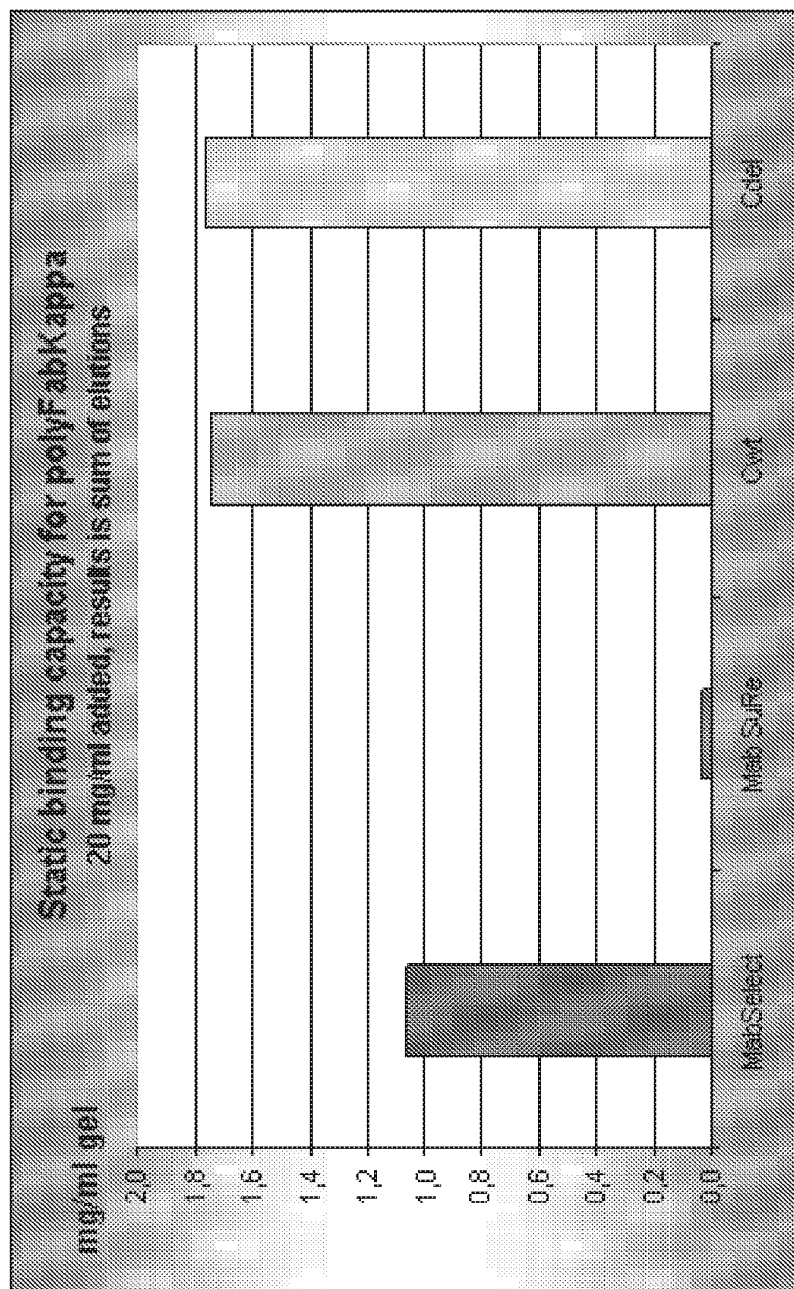


Figure 2

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**CHROMATOGRAPHY LIGAND
COMPRISING DOMAIN C FROM
STAPHYLOCOCCUS AUREUS PROTEIN A
FOR ANTIBODY ISOLATION**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 15/603,285, filed on May 23, 2017, which is a continuation of U.S. patent application Ser. No. 15/063,471, filed on Mar. 7, 2016, now U.S. Pat. No. 9,663,559, which is a division of U.S. patent application Ser. No. 14/164,519, filed Jan. 27, 2014, now U.S. Pat. No. 9,290,549, which is a continuation of U.S. patent application Ser. No. 13/559,663, filed Jul. 27, 2012, now U.S. Pat. No. 8,772,447, which is a division of U.S. patent application Ser. No. 12/443,011 filed on Mar. 26, 2009, and now U.S. Pat. No. 8,329,860, which This application is a filing under 35 U.S.C. § 371 and claims priority to international patent application number PCT/SE2007/000862 filed Sep. 27, 2007, published on Apr. 3, 2008, as WO 2008/039141, which claims priority to patent application number 0602061-4 filed in Sweden on Sep. 29, 2006.

STATEMENT REGARDING SEQUENCE
LISTING

The Sequence Listing associated with this application is part of the description and is provided in text the form of an Annex C/ST.25 text file in lieu of the text file containing the Sequence Listing is 220662-19 PU06101 CON DIV1CON_Sequence Listing.txt. The text file is 2 kb, was created on May 23, 2017, and is being submitted herewith electronically via EFS-Web.

FIELD OF THE INVENTION

The present invention relates to the field of chromatography, and more specifically to a novel affinity ligand which is suitable for use in antibody isolation. Thus, the invention encompasses affinity ligands as such, a chromatography matrix comprising ligands according to the invention, and a process of antibody isolation, wherein the ligand according to the invention is used.

BACKGROUND OF THE INVENTION

The term chromatography embraces a family of closely related separation methods based on the contacting of two mutually immiscible phases, wherein one phase is stationary and the other phase is mobile. One area wherein chromatography is of great interest is in the biotechnological field, such as for large-scale economic production of drugs and diagnostics. Generally, proteins are produced by cell culture, either intracellularly or by secretion into the surrounding. Since the cell lines used are living organisms, they must be fed with a complex growth medium containing sugars, amino acids, growth factors, etc. Separation of the desired protein from the mixture of compounds fed to the cells and from other cellular components to a sufficient purity, e.g. for use as a human therapeutic, poses a formidable challenge.

In such separation, in a first step, cells and/or cell debris is usually removed by filtration. Once a clarified solution containing the protein of interest has been obtained, its separation from the other components of the solution is often performed using a combination of different chromatography

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steps, often based on different separation principles. Thus, such steps separate proteins from mixtures on the basis of charge, degree of hydrophobicity, affinity properties, size etc. Several different chromatography matrices, such as matrices for ion exchange, hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC), affinity chromatography and immobilized metal affinity chromatography (IMAC), are available for each of these techniques, allowing tailoring of the purification scheme to the particular protein involved. An illustrative protein, which is of steadily growing interest in the medical field, is immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG).

As in all process technology, an important aim is to keep the production costs low. Consequently, improved chromatographic techniques are frequently presented, and the matrices are when possible reused. However, since each use of a chromatography matrix will leave certain traces of the operation just performed, many different cleaning protocols are available for cleaning and/or restoring the matrix into its original form. Commonly known materials that need to be removed are e.g. non-eluted proteins and protein aggregates as well as potentially hazardous materials, such as virus, endotoxins etc, which may originate from the cell culture. The most commonly used cleaning is a simple wash with buffer. For a more efficient cleaning of the matrix, treatments with acid and/or base are frequently used. In order to even more efficiently restore the matrix, an alkaline protocol known as Cleaning In Place (CIP) is commonly used. The standard CIP involves treatment of the matrix with 1M NaOH, pH 14. Such harsh treatment will efficiently remove undesired fouling of the above-discussed kind, but may in addition impair some chromatography matrix materials. For example, many affinity matrices, wherein the ligands are proteins or protein-based, cannot withstand standard CIP, at least not while maintaining their original properties. It is known that structural modification, such as deamidation and cleavage of the peptide backbone, of asparagine and glutamine residues in alkaline conditions is the main reason for loss of activity upon treatment of protein in alkaline solutions, and that asparagine is the most sensitive of the two. It is also known that the deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times in proteins have been associated with the sequences-asparagine-glycine- and -asparagine-serine. See e.g. Gülich, Linhult, Nygren, Uhlen and Hober (2000) Journal of Biotechnology 80, 169-178. Stability towards alkaline conditions can be engineered into a protein ligand.

Despite the documented alkaline sensitivity, protein A is widely used as a ligand in affinity chromatography matrices due to its ability to bind IgG without significantly affecting the affinity of immunoglobulin for antigen. As is well known, Protein A is a constituent of the cell wall of the bacterium *Staphylococcus aureus*. Such *Staphylococcus* protein, known as SpA, is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind antibodies at the Fc region, and a C-terminal region (or "X" region) that does not bind any antibodies. Jansson et al (Jansson, Uhlen and Nygren (1998) FEMS Immunology and Medical Microbiology 20, 69-78: "All individual domains of staphylococcal protein A show Fab binding") have later shown that all the individual SpA domains also bind certain antibodies at the Fab region.

U.S. Pat. No. 5,151,350 (Repligen) relates to cloning and expression of the gene coding for a protein A and protein A-like material. The cloning of this gene with its nucleotide sequence characterization enabled in 1982 for the first time

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to obtain quantities of a protein A-like material and nucleotide sequence for cloning in various host-vector systems.

Since the production of protein A in a recombinant system was accomplished, further genetic manipulations thereof have been suggested. For example, U.S. Pat. No. 5,260,373 (Repligen) describes genetic manipulation of recombinant protein A in order to facilitate the attachment thereof to a support, and more specifically to the coupling thereof via arginine. Further, U.S. Pat. No. 6,399,750 (Pharmacia Biotech AB) describes another recombinant protein A ligand, which has been coupled to a support via cysteine.

However, in order to maintain selectivity and binding capacity, Protein A chromatography matrices of the above-discussed kind need to be cleaned under milder conditions than conventional CIP. In this context, it is understood that the cleaning is closely related to the lifetime of the chromatography matrix. For example, a sensitive matrix may be cleaned with standard CIP, if a reduced performance is acceptable. Thus, efforts have been made to provide chromatography matrices which present the outstanding properties, such as selectivity, of protein A, but which are more resistant to alkaline conditions used for CIP.

Thus, U.S. Pat. No. 6,831,161 (Uhlén et al) relates to methods of affinity separation using immobilized proteinaceous affinity ligands, wherein one or more asparagine (Asn) residues have been modified to increase alkaline stability. This patent also describes methods of making a stabilized combinatorial protein by modification of Asn residues within a protein molecule to increase stability of the protein in alkaline conditions, and randomization of a protein molecule to modify its binding characteristics, and combinatorial proteins wherein in a step separate from the randomization step, the stability of the protein in alkaline conditions has been increased by modifying one or more of its Asn residues.

Further, WO 03/080655 (Amersham Biosciences) relates to an immunoglobulin-binding protein, wherein at least one asparagine residue has been mutated to an amino acid other than glutamine or aspartic acid. According to this patent application, such more specific mutation confers an increased chemical stability at pH-values of up to about 13-14 compared to the parental molecule. The mutated protein can for example be derived from a protein capable of binding to other regions of the immunoglobulin molecule than the complementarily determining regions (CDR), such as protein A, and preferably from the B-domain of Staphylococcal protein A. The invention also relates to a matrix for affinity separation, which comprises the described mutated immunoglobulin-binding proteins as ligands.

Despite the above-described development of more alkaline-stable protein A-based chromatography ligands, there is still a need in this field of improved ligands and chromatography matrices for highly specific isolation of antibodies, and of alternative wild type ligand constructions that allow easier manufacture.

One example of such an improved chromatography matrix is described in US 2006/0134805 (Berg et al), which relates to a separation matrix comprised of porous particles to which antibody-binding protein ligands have been immobilised. More specifically, the disclosed chromatography matrix has been optimised in terms of ligand density; gel phase distribution coefficient (K_{av}); and particle size to provide a matrix especially suitable for high capacity purification of antibodies. The ligands of the disclosed matrix may comprise antibody-binding protein such as Protein A, Protein G and/or Protein L.

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SUMMARY OF THE INVENTION

One aspect of the present invention is to provide a novel chromatography ligand, which is capable of withstanding repeated cleaning-in-place cycles. This may be achieved by an affinity ligand which is based on domain C from SpA Domain C, as defined in the appended claims.

Another aspect of the present invention is to provide an economical process of purifying immunoglobulins. This may be achieved by a process which uses an affinity chromatography ligand capable of withstanding repeated cleaning-in-place cycles.

Further aspects and advantages of the invention will appear from the detailed disclosure that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows results of testing the alkaline-stability of the ligand according to the invention as compared to other protein-based ligands. The X axis shows the incubation time in hours; while the Y axis shows the capacity that remains after X hours in 0.5M NaOH, as described in Example 1. More specifically, the Protein A-containing product MAB-SELECT™ (◆); the more recent Protein A product MAB-SELECT SURE™, marketed as more alkaline-stable (X); Domain C from SpA as defined by SEQ ID NO 1 (Δ); and finally a deleted embodiment of Domain C from SpA as defined by SEQ ID NO 2 (■). As appears from FIG. 1, the Domain C ligand according to the invention shows an alkaline-stability well comparable to the alkaline-stable product MABSELECT SURE™.

FIG. 2 shows the results of testing the Fab-binding properties of the ligand according to the invention, as compared to other protein-based ligands. As appears from this figure, a chromatography ligand comprising Domain C from SpA (Cwt and Cdel) present a much higher levels of Fab-binding than the other tested ligands.

DEFINITIONS

The term Domain C or “functional fragments or variants thereof” encompasses fragments or variants of SpA Domain C, which have the property of binding to IgG at the Fc region.

The terms “antibody” and “immunoglobulin” are used interchangeably herein, and are understood to include also fusion proteins comprising antibodies and fragments of antibodies.

The term an “Fc-binding protein” means a protein capable of binding to the crystallisable part (Fc) of an antibody and includes e.g. Protein A and Protein G, or any fragment or fusion protein thereof that has maintained said binding property.

The term “Fab fragment” refers to the variable part of an antibody; hence a “Fab-binding ligand” is capable of binding to either full antibodies via Fab-binding; or to antibody fragments which includes the variable parts also known as Fab fragments.

The term “chromatography” is used herein for any kind of separation which utilises the principles of chromatography, and hence includes batch as well as HPLC methods.

The term “affinity chromatography” is used herein for the specific mode of chromatography where the ligand interacts with target via biological affinity in a “lock-key” fashion. Examples of useful interactions in affinity chromatography are e.g. enzyme-substrate interaction, biotin-avidin interaction, antibody-antigen interaction etc.

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The term “protein-based” ligands means herein ligands which comprise a peptide or protein; or a part of a peptide or a part of a protein.

The term “isolation” of an antibody is used herein as embracing purification of a specific product antibody from a mixture comprising other proteins, such as other antibodies, and other components; as well as the separation of an antibody from a product liquid, i.e. to remove an undesired antibody.

DETAILED DESCRIPTION OF THE INVENTION

Thus, the present invention relates to a novel chromatography ligand. The chromatography ligand according to the invention, which is protein-based and of the kind known as affinity ligand, comprises all or parts of Domain C from *Staphylococcus* protein A (SpA). In a first aspect, the present invention relates to a chromatography ligand, which ligand comprises one or more Domain C units from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof. In one embodiment, the present chromatography ligand is substantially alkaline-stable. In this context, the term “substantially alkaline-stable” is understood to mean that the ligand is capable of withstanding repeated cleaning-in-place cycles using alkaline wash liquid without losing its binding capacity.

In a specific embodiment, the present invention is a chromatography ligand, comprises Domain C from *Staphylococcus* protein A (SpA), but none of the other domains of SpA.

In an alternative aspect, the present invention relates to a chromatography ligand, which ligand comprises one or more Domain C units from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof, which chromatography ligand is capable of binding to the Fab part of antibodies, as discussed in more detail below.

As discussed above, Jansson et al have already shown that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A. The present inventors have confirmed that the immunoglobulin binding properties of Domain C are fully satisfactory for the use thereof as a chromatography ligand. As also discussed above, Güllich and others had shown that asparagine and glutamine residues in alkaline conditions is the main reason for loss of protein A activity upon treatment in alkaline solutions, and that asparagine is the most sensitive of the two. Consequently, the Domain C ligand, which contains as many as six asparagine residues, was not be expected to present any substantial alkaline-stability as compared to protein A.

However, as shown in the experimental part below, and in FIG. 1, the present inventors have quite surprisingly shown that the SpA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product (MABSELECT™, GE Healthcare, Uppsala, Sweden) by incubation in alkaline conditions for durations as long as 20 hours. In fact, the Domain C ligand presents values of alkaline-stability which are similar to those of the product marketed as alkaline-stable (MABSELECT SURE™, GE Healthcare, Uppsala, Sweden), wherein asparagine residues have been mutated to other amino acids.

In addition to this, as discussed above, it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences-asparagine-glycine- and -asparagine-serine. Quite surprisingly, the Domain C ligand of the invention

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presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29, using the conventional numbering of the residues of Domain C.

In one embodiment, the ligand according to the invention is able to resist at least 10 hours in 0.5 M NaOH, without deviating more than about 10%, and preferably no more than 5%, from its original immunoglobulin binding capacity. Thus, after 5 hours, it will not deviate more than 10%, preferably 5% from its original binding capacity. In other words, one embodiment of the present invention is a ligand as described above, which after 5 hours incubation in 0.5M NaOH has retained at least 95% of its original binding capacity.

In an advantageous embodiment, the ligand according to the invention is able to resist at least 15 hours in 0.5 M NaOH without losing more than about 20%, and preferably no more than 10%, of its original immunoglobulin binding capacity. In a more advantageous embodiment, the ligand according to the invention is able to resist at least 20 hours in 0.5 M NaOH without losing more than about 30%, and preferably no more than 15%, of its original immunoglobulin binding capacity. In other words, one embodiment of the present invention is a ligand as described above, which after 15 hours incubation in 0.5 M NaOH has retained at least 80%, advantageously at least 90% of its original binding capacity.

The skilled person in this field can easily test alkaline-stability by incubating a candidate ligand with sodium hydroxide e.g. as described in the experimental part, and subsequent testing of the binding capacity by routine chromatography experiments.

As easily realised by the skilled person in this field, a chromatography ligand according to the invention may consist of the wild type SpA Domain C amino acid sequence, as shown in SEQ ID NO 1, herein denoted Cwt. In an alternative embodiment, the chromatography ligand according to the invention consists of a functional fragment of SpA Domain C, such as the one shown in SEQ ID NO 2, which discloses a sequence herein denoted. Cdel, wherein Asn-Lys-Phe-Asn in positions 3-6 have been deleted as compared to the wild type SpA Domain C sequence. In yet an alternative embodiment, a variant of SpA Domain C is prepared by adding one or more amino acids e.g. to either end of the wild type SpA Domain C amino acid sequence; or by mutation of the wild type SpA Domain C amino acid sequence, provided that such mutation does not substantially interfere with the herein described properties relating to immunoglobulin-binding and alkaline-stability. Thus, in a specific embodiment, the chromatography ligand according to the invention comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A. Alternatively, the chromatography ligand according to this embodiment comprises the deleted SpA Domain C, as shown in SEQ ID NO 2, which consequently comprises said mutation in position 25 (i.e. G25A). As the skilled person will recognise, such addition, mutation or deletion of amino acids as compared to the wild type sequence should preferably not substantially affect the folding pattern of the SpA Domain C ligand.

Thus, in one embodiment, the amino acid sequence of the ligand according to the present invention is the sequence defined by SEQ ID NO 1. In a specific embodiment, the ligand according to the invention comprises at least 60%, advantageously at least 80%, more advantageously at least 90% and most advantageously at least 95%, such as about 98% of the amino acids shown in SEQ ID NO 1. In a specific

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embodiment, the ligand according to the invention comprises at least 35, advantageously at least 46, more advantageously at least 52 and most advantageously at least 55, such as 57, of the amino acids shown in SEQ ID NO 1.

In an alternative embodiment, the amino acid sequence of the ligand according to the present invention is the sequence defined by SEQ ID NO 2. In a specific embodiment, the ligand according to the invention comprises at least 40%, advantageously at least 77%, more advantageously at least % and most advantageously at least 94%, such as about 98% of the amino acids shown in SEQ ID NO 2. In a specific embodiment, the ligand according to the invention comprises at least 31, advantageously at least 42, more advantageously at least 48 and most advantageously at least 51, such as 53, of the amino acids shown in SEQ NO 2.

As discussed in the section Background above, methods are readily available for coupling of protein ligands via certain amino acids, preferably amino acids that contain nitrogen and/or sulphur atoms, see e.g. U.S. Pat. No. 6,399, 750 or 5,084,559. Thus, in one embodiment, the ligand according to the invention further comprises a terminal coupling group, said group preferably comprising one or more nitrogen and/or sulphur atoms. In an advantageous embodiment, the terminal coupling group is comprised of arginine or cysteine. In one embodiment, the coupling group is in the C terminal region.

Further, the present invention also relates to a multimeric chromatography ligand (also denoted a "multimer") comprised of at least two Domain C units, or a functional fragments or variants thereof, as defined above. In one embodiment, this multimer comprises no units originating from SpA. In a specific embodiment, the multimer comprises no other protein-based units. In another embodiment, the multimer comprises no other unit capable of any substantial interaction with a target such as an antibody or a Fab fragment, thus it comprises no other ligand unit. As the skilled person in this field will realise, making a multimer may require adding one or more peptides as linkers between the units. Thus, a multimer limited to containing only Domain C units according to the invention may in addition comprise linkers allowing construction of a multimer wherein each Domain C unit is sufficiently exposed to be able to participate in the binding of target.

In another embodiment, the multimer comprises one or more additional units, which are different from Domain C and preferably protein-based and equally alkaline-stable as Domain C. Thus, in the multimer, the ligand according to the invention may be repeated and/or combined with other units from other sources, such as other proteins. In one embodiment, the multimer is comprised of 2-8 units, such as 4-6 units. In one embodiment, one or more linker sequences are inserted between the multimer units. Such linkers may e.g. be inserted to allow the actual ligand units to maintain their folding pattern. Linkers in this context are well known, and the skilled person can easily decide on suitable amino acids and chain lengths which do not interfere with the herein discussed properties of the ligand. In a specific embodiment, the chromatography ligand according to the invention comprises no other SpA domains than Domain C.

In a second aspect, the present invention relates to a nucleic acid sequence encoding a chromatography ligand as described above. Thus, the invention encompasses all forms of the present nucleic acid sequence such as the RNA and the DNA encoding the ligand. The invention embraces a vector, such as a plasmid, which in addition to the coding sequence comprises the required signal sequences for expression of the ligand according to the invention. In one embodiment, the

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vector comprises nucleic acid encoding a multimeric ligand according to the invention, wherein the separate nucleic acids encoding each unit may have homologous or heterologous DNA sequences. This aspect also embraces an expression system comprising a nucleic acid sequence encoding a ligand according to the invention. The expression system may e.g. be a prokaryotic host cell system, e.g. *E. coli* which has been modified to express the present ligand. In an alternative embodiment, the expression system is a eukaryotic host cell system, such as a yeast.

As the skilled person in this field will appreciate, the ligand according to the invention may alternatively be produced by protein synthesis methods, wherein the ligand is obtained by an automated process adding amino acids one at a time following a predetermined sequence. In an advantageous embodiment, segments of amino acids amino acid sequences are synthesized and linked to each other to prepare the ligand according to the invention. Such synthesis and linking procedures are well known to the skilled person in this field.

In a third aspect, the present invention relates to a chromatography matrix comprised of ligands as described above coupled to an insoluble carrier. Such a carrier may be one or more particles, such as beads or irregular shapes; membranes; filters; capillaries; monoliths; and any other format commonly used in chromatography. Thus, its an advantageous embodiment of the matrix, the carrier is comprised of substantially spherical particles, also known as beads. Suitable particle sizes may be in the diameter range of 5-500 μm , such as 10-100 μm , e.g. 20-80 μm . In an alternative embodiment, the carrier is a membrane. To obtain high adsorption capacities, the carrier is preferably porous, and ligands are then coupled to the external surfaces as well as to the pore surfaces. Thus, in an advantageous embodiment of the matrix according to the invention, the carrier is porous.

The carrier may be made from an organic or inorganic material. In one embodiment, the carrier is prepared from a native polymer, such as cross-linked carbohydrate material, e.g. agarose, agar, cellulose, dextran, chitosan, konjac, carrageenan, gellan, alginate etc. The native polymer carriers are easily prepared and optionally cross-linked according to standard methods, such as inverse suspension gelation (S Hjertén: *Biochim Biophys Acta* 79(2), 393-398 (1964)). In an alternative embodiment, the carrier is prepared from a synthetic polymer or copolymer, such as cross-linked synthetic polymers, e.g. styrene or styrene derivatives, divinylbenzene, acrylamides, acrylate esters, methacrylate esters, vinyl esters, vinyl amides etc. Such synthetic polymer carriers are easily prepared and optionally cross-linked according to standard methods, see e.g. "Styrene based polymer supports developed by suspension polymerization" (R Arshady: *Chimica L'Industria* 70(9), 70-75 (1988)). Native or synthetic polymer carriers are also available from commercial sources, such as GE Healthcare Bio-Sciences AB, Uppsala, Sweden, for example in the form of porous particles. In yet an alternative embodiment, the carrier is prepared from an inorganic polymer, such as silica. Inorganic porous and non-porous carriers are well known in this field and easily prepared according to standard methods.

In a fourth aspect, the present invention relates to a method of preparing a chromatography matrix, which method comprises providing ligands as described above; and coupling of said ligands to a carrier. In an advantageous embodiment, the coupling is carried out via a nitrogen or sulphur atom of the ligand. In brief, the ligands may be coupled to the carrier directly; or indirectly via a spacer

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element to provide an appropriate distance between the carrier surface and the ligand. Methods for immobilisation of protein ligands to porous or non-porous surfaces are well known in this field; see e.g. the above-discussed U.S. Pat. No. 6,399,750.

In a fifth aspect, the present invention relates to a process of isolating one or more target compounds, which process comprises contacting a liquid comprising said compound(s) with a chromatography matrix; allowing said compound(s) to adsorb to ligands present on the matrix, wherein said ligands consists of one or more *Staphylococcus* protein A (SpA) Domain C, and/or functional fragments or variants thereof; and, optionally, eluting said compound(s) by the passing across said matrix of a liquid that releases compound(s) from ligands. Thus, in this embodiment, the ligands comprise no other. SpA-derived domain than Domain C, or a functional fragment or variant thereof. In an alternative embodiment, said ligands are multimers comprising two or more SpA Domain C units, or functional fragments or variants thereof.

In an advantageous embodiment, the ligands are the ligands described above. The target compound(s) may be any organic compound, biomolecule or other biological material, such as proteins, e.g. antibodies; peptides; cells, such as eukaryotic and prokaryotic cells; nucleic acids, such as DNA, e.g. plasmids, and RNA; virus; etc. In an advantageous embodiment, the target compound(s) is one or more monoclonal or polyclonal antibodies, such as IgA, IgD, IgE, IgG, and IgM. In one embodiment, the target compound is a fragment of an antibody, such as a Fab fragment. In yet another embodiment, the target compound is a fusion protein wherein at least one part is an antibody or an antibody fragment.

In one embodiment, the chromatography matrix is a disposable product, and elution will then not be required if the purpose of the process is to remove the target compound such as the antibody from a product liquid. This embodiment may e.g. be for the removal of an undesired antibody from a liquid, such as a medical liquid or a liquid wherein many antibodies are produced, such as milk from a recombinant animal.

In an alternative embodiment, when the adsorbed compound is the desired product, the elution step is included in the process. To obtain the most suitable conditions for adsorption, a liquid sample is combined with a suitable buffer or other liquid such as water to provide the mobile phase. The present method is advantageously run under conditions conventional for affinity chromatography, and especially for protein A chromatography, as is well known in this field.

In a sixth aspect, the present invention relates to the use Domain C of SpA, or a functional fragment or variant thereof, as alkaline-stable immunoglobulin adsorbent. In this context, "alkaline-stable" is understood to mean that the adsorbent alkaline-stability is not lower than about 10%, such as about 5%, below that of a commercial products marketed as being alkaline-stable, such as MABSELECT SURE™ (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) during the first 5 hours of incubation in 0.5M NaOH. In an advantageous embodiment, the adsorbent is a ligand as described above. As said MABSELECT SURE™ should present a minimal deterioration after such time and conditions, the antibody binding capacity of the adsorbent should not be lower than about 10%, such as about 5%, below its original binding capacity after such time and conditions. In this context, the term "original" refers to its capacity before

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any alkaline regeneration, and the comparisons are carried out as side-by-side experiments using a procedure of the herein disclosed kind.

In one embodiment, the use according to the invention comprises a process as described above, wherein the antibodies are eluted from the matrix and which is carried out at least once, such as 2-300 times, optionally with washing steps between; alkaline regeneration of the matrix; and finally repeating said process of isolating antibodies. Washing may e.g. be carried out with a suitable buffer, such as the buffer used to equilibrate the column. In an advantageous embodiment, the regeneration is carried out by incubation with 0.5 M NaOH.

The present invention also embraces a method of purifying one or more target compounds, as discussed above, which method comprises one or more chromatography steps in addition to the purification using the chromatography matrix according to the invention. The method according to this aspect may e.g. comprises first chromatography step using the present matrix; an intermediate chromatography step using either ion exchange or hydrophobic interaction chromatography (HIC); and finally a polishing step using ion exchange, HIC or reverse phase chromatography. In a specific embodiment, this process comprises a step preceding the chromatography matrix having Domain C ligands as described herein. Such a preceding step may e.g. be a conventional filtration, sedimentation, flocculation or other step to remove cell debris and other undesired components.

In an alternative embodiment, the use according to the invention is an analytical or diagnostic use, such as an immunoassay.

EXAMPLES

The present examples are provided as illustrative purposes only, and should not be construed as limiting the present invention as defined in the appended claims.

Example 1: Column Study of the Alkaline Stability of Four Protein A-Derived Ligands

In this example, the alkaline stability of four chromatography matrices, two of which were comparative and two of which were according to the invention, were tested through a series of chromatographic runs:

MABSELECT™ and MABSELECT SURE™ (both comparative products comprising protein-based ligands, GE Healthcare Bio-Sciences, Uppsala, Sweden), and

Cwt (wild type Domain C from SpA, as defined in SEQ NO. 1), and Cdel (deleted wild type Domain C from SpA, as defined in SEQ ID NO. 2).

The IgG-binding capacity was measured initially and after incubation steps in 0.5 M NaOH. The incubation times varied from one to five hours, with an accumulated incubation time of 20 hours.

The ligands according to the invention were immobilized on agarose particles according to standard procedure and packed in columns (GE Healthcare). Two of the matrices, MABSELECT™ and MABSELECT SURE™, are commercial products manufactured by GE Healthcare marketed for the purification of monoclonal antibodies. The ligands of both products are based on the IgG binding *Staphylococcus aureus* Protein A. The MABSELECT™ ligand basically is recombinant Protein A, which consists of five homologous domains (E, D, A, B, C). By comparison, the MABSELECT SURE™ ligand consists of four domains which originate from the domain B analogue "Z", which in turn has been

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stabilized against high by protein engineering methods. As a result, MABSELECT SURE™ tolerates cleaning-in-place (CIP) conditions of up to 0.5 M NaOH. Both the MABSELECT™ and MABSELECT SURE™ ligands are coupled to agarose particles.

The ligands Cwt and Cdel were constructed as tetramers of identical domains with a C-terminal cysteine residue for coupling to a matrix according to standard procedure.

Materials & Methods

Target Compound

10×10 ml injection liquid, solution, GAMMANORM® 165 mg/ml (Octapharma no. 00 86 64), human normal immunoglobulin, for subcutane infusion or intramuscular injection, was used as the target compound in the chromatography experiments.

Chromatography Columns

Ligand coupling and column packing was carried out as outlined in Table 1 below:

TABLE 1

Columns used in Experiment 1					
Ligand/Matrix	Column ID	Column no.	Batch	Date	Column volume (ml)
MABSELECT SURE™	9	4	U669082	2006 Mar. 10	2.08
Cwt	11	2	U1555055A	2006 Mar. 10	2.02
MABSELECT™	1	7	U1555045A	2006 Mar. 10	2.12
Cdel	13	2	U1555059A	2006 Mar. 3	2.06

“Column ID” refers to a unique number given to each column. These numbers were included in the chromatography methods and can be found in the logbook of the result files. For example, the first column in table 1 was called “MABSELECT SURE™ U669082 Column 4 20060310 (9)”. “Column no.” is the packing number, i.e. columns packed with the same batch of matrix received different Column nos. upon packing. The column volume was estimated by measuring the bed height.

Buffers and Solutions

Buffer A: 50 mM Sodium phosphate, 0.15 M NaCl, pH 7.2

Buffer B: 50 mM Citric acid, 0.15 M NaCl, pH 2.5

Instruments and Laboratory Equipment

Chromatography system: ÄKTA EXPLORER™ 10 (GE Healthcare)

Column hardware: TRICORN™ 5/100 GL (GE Healthcare)

Vacuum degasser: CT 2003-2, 2 channel degasser, ChromTech AB

Spectrophotometer: NANODROP™ ND-1000 Spectrophotometer, NanoDrop Technologies

Centrifuge: Beckman Coulter AVANTI® J-20 XPI with JLA 8.1000 rotor

pH meter (Buffer A): Beckman Φ360 pH/Temp/mV Meter

pH meter (Buffer B): Laboratory pH Meter CG 842, SCHOTT

Helium: AGA Gas AB, 10 1H 20577708, Instrument

Filter for buffer and sample: 75 mm Bottle Top Filter—500 ml, 0.2 μm pore size, Nalgene

Filter for 0.5 M NaOH: 75 mm Bottle Top Filter—500 ml, 0.45 μm pore size, Nalgene

Software

ÄKTA EXPLORER™ 10 was controlled by UNICORN™ 5.01 (GE Healthcare). Apart from controlling the

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system during the chromatography runs, UNICORN™ was used for method programming and evaluation of the results.

Buffer Preparation

Buffer A: Sodium dihydrogen phosphate and NaCl were dissolved in water. A pH meter was calibrated using pH 4, pH 7 and pH 10 standard buffers. pH was monitored adding NaOH(aq) to the buffer until pH reached 7.2. The buffer was filtered and degassed with helium prior use.

Buffer B: Citric acid and NaCl were dissolved in water. A pH meter was calibrated using pH 7 and pH 2 standard buffers. pH was monitored while adding NaOH(aq) to the buffer until pH reached 2.5. The buffer was filtered and degassed with helium prior use.

Preparation of 0.5 M NaOH

NaOH(s) was dissolved in water to 0.5 M. The solution was filtered and degassed with helium prior use.

Sample Preparation

Experiment 1

30 ml Gammanorm (165 mg/ml) was diluted to 1 mg/ml with 4950 ml Buffer A. The sample was filtered through 0.2 μm into a sterile 5 liter bottle.

Three 280 nm absorbance measurements were performed on the sample using NANODROP™ spectrophotometer: 1.2573 AU, 1.2432 AU and 1.2101 AU. Mean absorbance: 1.2369 AU.

The absorbance at 280 nm was also measured on ÄKTA EXPLORER™ 10. The sample was pumped with the system pump through the system in bypass mode. A 10 mm UV cell was used and the flow rate was 0.83 ml/min. The absorbance at 280 nm was 1510 mAU. This value was used as a reference when making capacity calculations.

Method Description

Normally, a CIP cycle for MABSELECT SURE™ involves 10-15 minutes contact time of the CIP solution (usually 0.1-0.5 M NaOH). To reduce the amount of CIP cycles in this study, longer contact times were used. The columns were incubated for 1, 2 and 5 hour intervals, with a total contact time of 20 hours. This corresponds to 80 to 120 cycles with 10-15 minutes contact time.

Prior to the CIP incubations two initial capacity measurements were performed per column. After the capacity measurements the columns were incubated in 0.5 M NaOH. After each CIP incubation, one capacity measurement per column was carried out.

Schematically, the experiment was designed as follows:

- Two initial capacity measurements per column.
- CIP incubation, 1 hour.
- One capacity measurement per column.
- CIP incubation, 2 hour.
- One capacity measurement per column.
- CIP incubation, 2 hour.
- One capacity measurement per column.
- CIP incubation, 5 hour.
- One capacity measurement per column.
- CIP incubation, 5 hour.
- One capacity measurement per column.
- CIP incubation, 5 hour.
- One capacity measurement per column.
- System Setup:

The experiments were carried out in room temperature. However, the sample was kept on ice to avoid microbial growth. To avoid the formation of air bubbles when the cold sample was heated to room temperature, a degasser was

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connected between the sample and the pump. The ÄKTA EXPLORER™ 10 was equipped with a 10 mm UV cell for UV detection.

Both buffer and sample was pumped through the system pumps. Following inlets were used:

Sample: B pump (inlet B1)

Buffer A: A pump (inlet A11)

Buffer B: A pump (inlet A12)

0.5 M NaOH: A pump (inlet A13)

Capacity Measurement, Detailed Description

Prior to a capacity measurement (consisting of one capacity measurement per column) sample was pumped in bypass mode, i.e. no column used. The purpose of this was to get “fresh” sample to each capacity measurement and to avoid loading the first volume of sample that remained in tubes and the pump in room temperature during the CIP incubations, onto the first column.

The capacity measurement method for each column consisted of following parts:

Equilibration of the column with 5 column volumes (CV) Buffer A.

Sample loading. Dynamic binding capacity is determined by loading a sample onto a column packed with the chromatography medium of interest. When the medium becomes more and more saturated with sample, the level of absorbance at 280 nm will increase due to unbound sample passing through the column. In this method, the sample was loaded onto the column until the UV_{280 nm} curve reached 15% of the 280 nm absorbance of the sample.

Wash out unbound sample. The column was washed with Buffer A until the UV_{280 nm} curve dropped below 10% of the 280 nm absorbance of the sample

Elution. Bound material was eluted with 10 CV of Buffer B.

Reequilibration with 5 CV Buffer A.

The flow rate of sample loading was 0.83 ml/min.

CIP Incubation

After each capacity measurement, except for the first of the two initial measurements, a CIP incubation was carried out. In the CIP incubation method, 3 CV of 0.5 M NaOH was pumped through each column at a flow rate of 0.83 ml/min. After this the system was set to pause. The length of the pause depended on the length of the CIP incubation time, i.e. 1 h, 2 h or 5 h. However, the time required for the system to pump NaOH through the columns was subtracted from the pause time. After a CIP incubation 3 CV of Buffer A was pumped through each column at a flow rate of 0.83 ml/min to remove the NaOH. By this procedure, all columns were exposed the same amount of time to NaOH. One more wash cycle with 3 CV Buffer A was finally carried out.

Evaluation of Chromatographic Results

Capacity was determined by measuring the volume of sample applied onto a column until the absorbance at 280 nm reached 10% of the sample absorbance. The dead volumes, i.e. the column volume, mixer and tubing from the pump to the UV cell, were subtracted from this volume. The delay volume without column was determined to 1.02 ml. The capacity values were plotted against the accumulated CIP incubation times. Relative capacity values were achieved by dividing the capacity values after the CIP cycles with the mean of the start capacity values. The relative capacity values were used for easier comparisons between the different matrices.

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TABLE 2

Results Experiment 1 - Capacity (mg Gammanorm/ml chromatography matrix (gcl))

	MABSELECT SURE™	Cwt	MABSELECT™	Cdel
Start	28.38	27.43	28.98	30.86
Capacity 1				
Start	28.13	27.40	28.98	30.95
Capacity 2				
Capacity after 1 h	29.32	27.79	26.98	30.75
Capacity after 3 h	28.42	27.26	23.08	29.88
Capacity after 5 h	28.25	26.94	20.30	29.25
Capacity after 10 h	27.88	26.07	15.79	26.87
Capacity after 15 h	27.01	24.65	12.52	23.70
Capacity after 20 h	25.93	23.02	10.14	20.35

Experiment 2: Test of Fab-Binding

The Fab-binding ability of the different chromatography media was evaluated, in a 96-well filter plate assay. Liquids and chromatography media were mixed on a plate vortex instrument for 1 minute. The bottom of the wells consisted of a filter which retained liquids and the particles of the chromatography media. When subjected to centrifugation, the liquids passed through the filter and were collected in a separate 96-well collection UV-plate attached to the bottom of the filter plate. The absorbance at 280 nm of the collected liquid was measured in a plate reader and used for detection and estimation of Fab. The liquids from different steps, eg washing, elution, were collected in different plates and measured separately, to be able to measure the amount of Fab in individual fractions.

10% slurry was prepared of each chromatography medium.

The filter plates were loaded with 200 µl slurry/well, i.e. 20 µl medium/well.

Equilibration—5×200 µl wash in PBS

Sample incubation—100 µl of human polyclonal Fab/Kappa, IgG fragment (Bethyl) in PBS, 15 minutes

Wash—5×100 µl PBS

Elution—3×100 µl 0.1 M glycine, pH 3.0

CIP—2×10 min with 0.5 M NaOH

Analyze plates with liquids UV @280 nm

The results of experiment 2 are presented in FIG. 2.

The above examples illustrate specific aspects of the present invention and are not intended to limit the scope thereof in any respect and should not be so construed. Those skilled in the art having the benefit of the teachings of the present invention as set forth above, can effect numerous modifications thereto. These modifications are to be construed as being encompassed within the scope of the present invention as set forth in the appended claims.

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SEQUENCE LISTING

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<211> LENGTH: 58

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 1

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 1 5 10 15

Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
 20 25 30

Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
 35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
 50 55

<210> SEQ ID NO 2

<211> LENGTH: 54

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 2

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 1 5 10 15

Asn Leu Thr Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp
 20 25 30

Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala Lys Lys Leu Asn
 35 40 45

Asp Ala Gln Ala Pro Lys
 50

What is claimed is:

1. A process for isolating one or more target compound(s), 40 the process comprising:
 - (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and 45
 - (ii) at least one ligand coupled to the solid support, the ligand comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) 50 at a position corresponding to position 29 of SEQ ID NO. 1; and
 - (b) adsorbing the target compound(s) to the ligand; and,
 - (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand. 55
2. The process of claim 1, wherein the ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.
3. The process of claim 1, wherein the chromatography 60 matrix is capable of retaining at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.
4. The process of claim 1, wherein the ligand binds to the Fab part of an antibody.
5. The process of claim 1, wherein the ligand comprises 65 a terminal coupling group comprising at least one nitrogen and/or sulfur atom(s).
6. The process of claim 5, wherein the terminal group comprises arginine or cysteine.
7. The process of claim 1, wherein the ligand is coupled to the solid support via thioether bonds.
8. The process of claim 1, wherein the ligand further comprises one or more other alkaline-stable protein-based units.
9. The process of claim 1, wherein the solid support is a polysaccharide.
10. The process of claim 1, wherein the solid support is comprised of substantially spherical particles.
11. The process of claim 1, wherein the solid support is porous.
12. The process of claim 1, wherein the ligand comprises an amino acid sequence that comprises 2-8 of the polypeptides.
13. The process of claim 1, wherein two or more ligands are coupled to the solid support.
14. A process for isolating one or more target compound(s), the process comprising:
 - (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) a ligand coupled to the solid support, the ligand comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids in alignment with SEQ ID NO. 1, and wherein each polypeptide has an alanine

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- (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1;
 (b) adsorbing the target compound(s) to the ligand; and
 (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand.

15 **15.** The process of claim **14**, wherein the ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.

16. The process of claim **14**, wherein the chromatography matrix is capable of retaining at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.

17. The process of claim **14**, wherein the ligand binds to the Fab part of an antibody.

18. The process of claim **14**, wherein the ligand comprises a terminal coupling group comprising at least one nitrogen and/or sulfur atom(s).

19. The process of claim **18**, wherein the terminal group comprises arginine or cysteine.

20. The process of claim **14**, wherein the ligand is coupled to the solid support via thioether bonds.

21. The process of claim **14**, wherein the ligand further comprises one or more other alkaline-stable protein-based units.

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22. The process of claim **14**, wherein the solid support is a polysaccharide.

23. The process of claim **14**, wherein the solid support is comprised of substantially spherical particles.

24. The process of claim **14**, wherein the solid support is porous.

25. The process of claim **14**, wherein the ligand comprises an amino acid sequence that comprises 2-8 of the polypeptides.

26. The process of claim **14**, wherein two or more ligands are coupled to the solid support.

27. The process of claim **1**, further comprising a step of exposing the chromatography matrix to 0.1 to 0.5 M NaOH.

28. The process of claim **27**, further comprising repeated exposure of the chromatography matrix to the NaOH for at least 80 cycles.

29. The process of claim **14**, further comprising a step of exposing the chromatography matrix to 0.1 to 0.5 M NaOH.

30. The process of claim **29**, further comprising repeated exposure of the chromatography matrix to the NaOH for at least 80 cycles.

* * * * *



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(12) **United States Patent**
Hall et al.

(10) **Patent No.:** **US 10,875,007 B2**

(45) **Date of Patent:** **Dec. 29, 2020**

(54) **CHROMATOGRAPHY LIGAND
COMPRISING DOMAIN C FROM
STAPHYLOCOCCUS AUREUS PROTEIN A
FOR ANTIBODY ISOLATION**

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(30) **Foreign Application Priority Data**

Sep. 29, 2006 (SE) 0602061

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C07K 1/22 (2006.01)
B01J 20/32 (2006.01)
C07K 14/31 (2006.01)
C07K 16/00 (2006.01)
C07K 17/00 (2006.01)
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B01J 20/285 (2006.01)
B01J 20/289 (2006.01)
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CPC **B01J 20/24** (2013.01); **B01D 15/3809** (2013.01); **B01J 20/285** (2013.01); **B01J 20/286** (2013.01); **B01J 20/289** (2013.01); **B01J 20/28019** (2013.01); **B01J 20/3212** (2013.01); **B01J 20/3219** (2013.01); **B01J 20/3274** (2013.01); **B01J 20/3293** (2013.01); **C07K 1/22** (2013.01); **C07K 14/31** (2013.01); **C07K 16/00** (2013.01); **C07K 17/00** (2013.01); **C07K 17/10** (2013.01); **B01J 2220/52** (2013.01); **B01J 2220/54** (2013.01); **C07K 2317/55** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to a chromatography ligand, which comprises Domain C from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof. The chromatography ligand presents an advantageous capability of withstanding harsh cleaning in place (CIP) conditions, and is capable of binding Fab fragments of antibodies. The ligand may be provided with a terminal coupling group, such as arginine or cysteine, to facilitate its coupling to an insoluble carrier such as beads or a membrane. The invention also relates to a process of using the ligand in isolation of antibodies, and to a purification protocol which may include washing steps and/or regeneration with alkali.

37 Claims, 2 Drawing Sheets

Specification includes a Sequence Listing.

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Gulich, S. et al., "Stability towards alkaline conditions can be engineered into a protein ligand" *Journal of Biotechnology*, 80 (2000), pp. 169-178.

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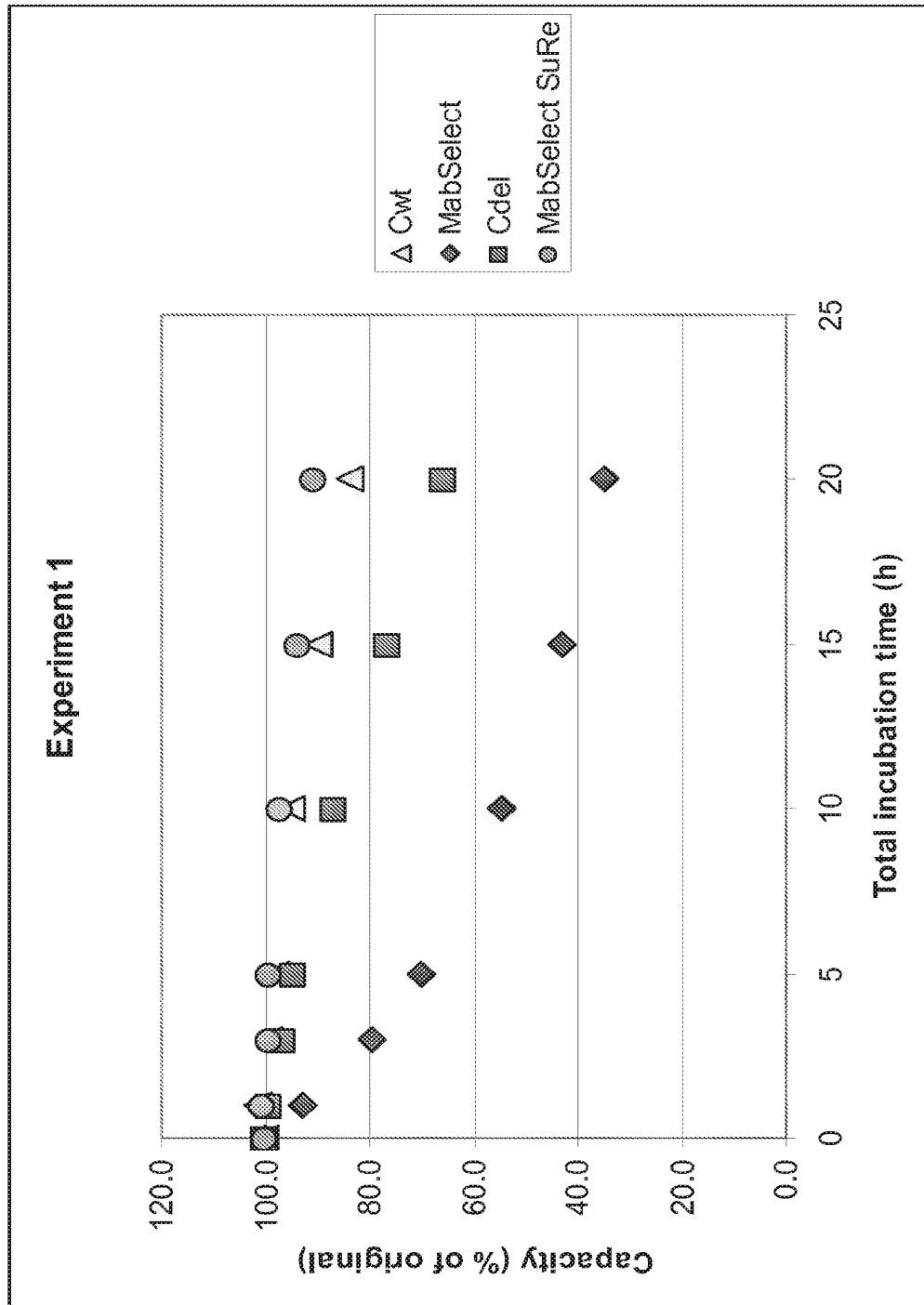


Figure 1

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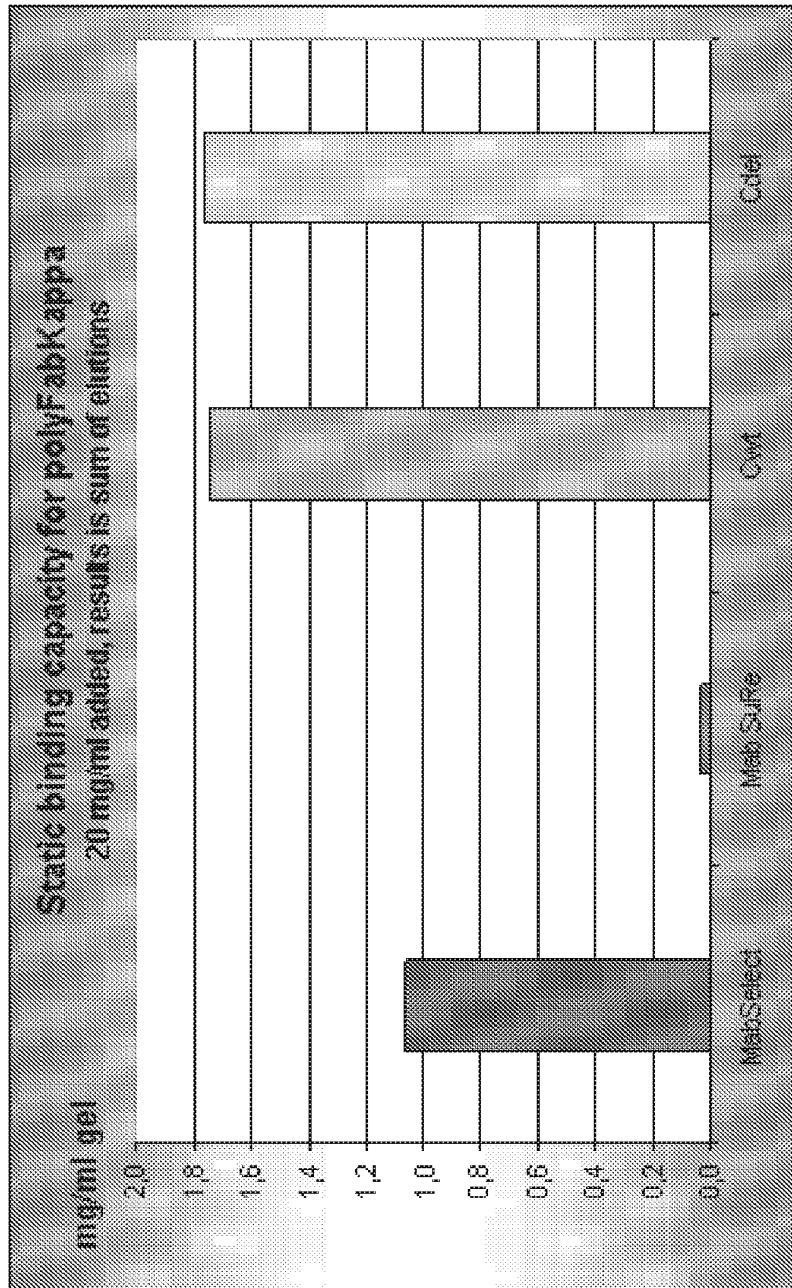


Figure 2

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**CHROMATOGRAPHY LIGAND
COMPRISING DOMAIN C FROM
STAPHYLOCOCCUS AUREUS PROTEIN A
FOR ANTIBODY ISOLATION**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 16/189,894, filed Nov. 13, 2018, which is a continuation of U.S. patent application Ser. No. 15/603,285, filed on May 23, 2017, which is a continuation of U.S. patent application Ser. No. 15/063,471, filed on Mar. 7, 2016, now U.S. Pat. No. 9,663,559, which is a division of U.S. patent application Ser. No. 14/164,519, filed Jan. 27, 2014, now U.S. Pat. No. 9,290,549, which is a continuation of U.S. patent application Ser. No. 13/559,663, filed Jul. 27, 2012, now U.S. Pat. No. 8,772,447, which is a division of U.S. patent application Ser. No. 12/443,011 filed on Mar. 26, 2009, and now U.S. Pat. No. 8,329,860, which This application is a filing under 35 U.S.C. § 371 and claims priority to international patent application number PCT/SE2007/000862 filed Sep. 27, 2007, published on Apr. 3, 2008, as WO 2008/039141, which claims priority to patent application number 0602061-4 filed in Sweden on Sep. 29, 2006, the entire disclosures of each of which are hereby incorporated by reference.

STATEMENT REGARDING SEQUENCE
LISTING

The Sequence Listing associated with this application is part of the description and is provided in text the form of an Annex C/ST.25 text file in lieu of the text file containing the Sequence Listing is 220662-19 PU06101 CON DIVICON_Sequence Listing.txt. The text file is 2 kb, was created on May 23, 2017, and is being submitted herewith electronically via EFS-Web.

FIELD OF THE INVENTION

The present invention relates to the field of chromatography, and more specifically to a novel affinity ligand which is suitable for use in antibody isolation. Thus, the invention encompasses affinity ligands as such, a chromatography matrix comprising ligands according to the invention, and a process of antibody isolation, wherein the ligand according to the invention is used.

BACKGROUND OF THE INVENTION

The term chromatography embraces a family of closely related separation methods based on the contacting of two mutually immiscible phases, wherein one phase is stationary and the other phase is mobile. One area wherein chromatography is of great interest is in the biotechnological field, such as for large-scale economic production of drugs and diagnostics. Generally, proteins are produced by cell culture, either intracellularly or by secretion into the surrounding medium. Since the cell lines used are living organisms, they must be fed with a complex growth medium containing sugars, amino acids, growth factors, etc. Separation of the desired protein from the mixture of compounds fed to the cells and from other cellular components to a sufficient purity, e.g. for use as a human therapeutic, poses a formidable challenge.

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In such separation, in a first step, cells and/or cell debris is usually removed by filtration. Once a clarified solution containing the protein of interest has been obtained, its separation from the other components of the solution is often performed using a combination of different chromatography steps, often based on different separation principles. Thus, such steps separate proteins from mixtures on the basis of charge, degree of hydrophobicity, affinity properties, size etc. Several different chromatography matrices, such as matrices for ion exchange, hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC), affinity chromatography and immobilized metal affinity chromatography (IMAC), are available for each of these techniques, allowing tailoring of the purification scheme to the particular protein involved. An illustrative protein, which is of steadily growing interest in the medical field, is immunoglobulin proteins, also known as antibodies, such as immunoglobulin G (IgG).

As in all process technology, an important aim is to keep the production costs low. Consequently, improved chromatographic techniques are frequently presented, and the matrices are when possible reused. However, since each use of a chromatography matrix will leave certain traces of the operation just performed, many different cleaning protocols are available for cleaning and/or restoring the matrix into its original form. Commonly known materials that need to be removed are e.g. non-eluted proteins and protein aggregates as well as potentially hazardous materials, such as virus, endotoxins etc, which may originate from the cell culture. The most commonly used cleaning is a simple wash with buffer. For a more efficient cleaning of the matrix, treatments with acid and/or base are frequently used. In order to even more efficiently restore the matrix, an alkaline protocol known as Cleaning In Place (CIP) is commonly used. The standard CIP involves treatment of the matrix with 1M NaOH, pH 14. Such harsh treatment will efficiently remove undesired fouling of the above-discussed kind, but may in addition impair some chromatography matrix materials. For example, many affinity matrices, wherein the ligands are proteins or protein-based, cannot withstand standard CIP, at least not while maintaining their original properties. It is known that structural modification, such as deamidation and cleavage of the peptide backbone, of asparagine and glutamine residues in alkaline conditions is the main reason for loss of activity upon treatment of protein in alkaline solutions, and that asparagine is the most sensitive of the two. It is also known that the deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times in proteins have been associated with the sequences—asparagine-glycine- and -asparagine-serine. See e.g. Gülich, Linhult, Nygren, Uhlen and Hober (2000) Journal of Biotechnology 80, 169-178. Stability towards alkaline conditions can be engineered into a protein ligand. Despite the documented alkaline sensitivity, protein A is widely used as a ligand in affinity chromatography matrices due to its ability to bind IgG without significantly affecting the affinity of immunoglobulin for antigen. As is well known, Protein A is a constituent of the cell wall of the bacterium *Staphylococcus aureus*. Such *Staphylococcus* protein, known as SpA, is composed of five domains, designated in order from the N-terminus as E, D, A, B, and C, which are able to bind antibodies at the Fc region, and a C-terminal region (or “X” region) that does not bind any antibodies. Jansson et al (Jansson, Uhlen and Nygren (1998) FEMS Immunology and Medical Microbiology 20, 69-78: “All individual domains of staphylococcal protein A show

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Fab binding”) have later shown that all the individual SpA domains also bind certain antibodies at the Fab region.

U.S. Pat. No. 5,151,350 (Repligen) relates to cloning and expression of the gene coding for a protein A and protein A-like material. The cloning of this gene with its nucleotide sequence characterization enabled in 1982 for the first time to obtain quantities of a protein A-like material and nucleotide sequence for cloning in various host-vector systems.

Since the production of protein A in a recombinant system was accomplished, further genetic manipulations thereof have been suggested. For example, U.S. Pat. No. 5,260,373 (Repligen) describes genetic manipulation of recombinant protein A in order to facilitate the attachment thereof to a support, and more specifically to the coupling thereof via arginine. Further, U.S. Pat. No. 6,399,750 (Pharmacia Biotech AB) describes another recombinant protein A ligand, which has been coupled to a support via cysteine.

However, in order to maintain selectivity and binding capacity, Protein A chromatography matrices of the above-discussed kind need to be cleaned under milder conditions than conventional CIP. In this context, it is understood that the cleaning is closely related to the lifetime of the chromatography matrix. For example, a sensitive matrix may be cleaned with standard CIP, if a reduced performance is acceptable. Thus, efforts have been made to provide chromatography matrices which present the outstanding properties, such as selectivity, of protein A, but which are more resistant to alkaline conditions used for CIP.

Thus, U.S. Pat. No. 6,831,161 (Uhlén et al) relates to methods of affinity separation using immobilized proteinaceous affinity ligands, wherein one or more asparagine (Asn) residues have been modified to increase alkaline stability. This patent also describes methods of making a stabilized combinatorial protein by modification of Asn residues within a protein molecule to increase stability of the protein in alkaline conditions, and randomization of a protein molecule to modify its binding characteristics, and combinatorial proteins wherein in a step separate from the randomization step, the stability of the protein in alkaline conditions has been increased by modifying one or more of its Asn residues.

Further, WO 03/080655 (Amersham Biosciences) relates to an immunoglobulin-binding protein, wherein at least one asparagine residue has been mutated to an amino acid other than glutamine or aspartic acid. According to this patent application, such more specific mutation confers an increased chemical stability at pH-values of up to about 13-14 compared to the parental molecule. The mutated protein can for example be derived from a protein capable of binding to other regions of the immunoglobulin molecule than the complementarily determining regions (CDR), such as protein A, and preferably from the B-domain of Staphylococcal protein A. The invention also relates to a matrix for affinity separation, which comprises the described mutated immunoglobulin-binding proteins as ligands.

Despite the above-described development towards more alkaline-stable protein A-based chromatography ligands, there is still a need in this field of improved ligands and chromatography matrices for highly specific isolation of antibodies, and of alternative wild type ligand constructions that allow easier manufacture.

One example of such an improved chromatography matrix is described in US 2006/0134805 (Berg et al), which relates to a separation matrix comprised of porous particles to which antibody-binding protein ligands have been immobilised. More specifically, the disclosed chromatography matrix has been optimised in terms of ligand density; gel

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phase distribution coefficient (Kav); and particle size to provide a matrix especially suitable for high capacity purification of antibodies. The ligands of the disclosed matrix may comprise antibody-binding protein such as Protein A, Protein G and/or Protein L.

SUMMARY OF THE INVENTION

One aspect of the present invention is to provide a novel chromatography ligand, which is capable of withstanding repeated cleaning-in-place cycles. This may be achieved by an affinity ligand which is based on domain C from SpA Domain C, as defined in the appended claims.

Another aspect of the present invention is to provide an economical process of purifying immunoglobulins. This may be achieved by a process which uses an affinity chromatography ligand capable of withstanding repeated cleaning-in-place cycles.

Further aspects and advantages of the invention will appear from the detailed disclosure that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows results of testing the alkaline-stability of the ligand according to the invention as compared to other protein-based ligands. The X axis shows the incubation time in hours; while the Y axis shows the capacity that remains after X hours in 0.5M NaOH, as described in Example 1. More specifically, the Protein A-containing product MAB-SELECT™ (◆); the more recent Protein A product MAB-SELECT SURE™, marketed as more alkaline-stable (X); Domain C from SpA as defined by SEQ ID NO 1 (Δ); and finally a deleted embodiment of Domain C from SpA as defined by SEQ ID NO 2 (■). As appears from FIG. 1, the Domain C ligand according to the invention shows an alkaline-stability well comparable to the alkaline-stable product MABSELECT SURE™.

FIG. 2 shows the results of testing the Fab-binding properties of the ligand according to the invention, as compared to other protein-based ligands. As appears from this figure, a chromatography ligand comprising Domain C from SpA (Cwt and Cdel) present a much higher levels of Fab-binding than the other tested ligands.

DEFINITIONS

The term Domain C or “functional fragments or variants thereof” encompasses fragments or variants of SpA Domain C, which have the property of binding to IgG at the Fc region. The terms “antibody” and “immunoglobulin” are used interchangeably herein, and are understood to include also fusion proteins comprising antibodies and fragments of antibodies.

The term an “Fc-binding protein” means a protein capable of binding to the crystallisable part (Fc) of an antibody and includes e.g. Protein A and Protein G, or any fragment or fusion protein thereof that has maintained said binding property.

The term “Fab fragment” refers to the variable part of an antibody; hence a “Fab-binding ligand” is capable of binding to either full antibodies via Fab-binding; or to antibody fragments which includes the variable parts also known as Fab fragments.

The term “chromatography” is used herein for any kind of separation which utilises the principles of chromatography, and hence includes batch as well as HPLC methods. The term “affinity chromatography” is used herein for the spe-

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cific mode of chromatography where the ligand interacts with target via biological affinity in a “lock-key” fashion. Examples of useful interactions in affinity chromatography are e.g. enzyme-substrate interaction, biotin-avidin interaction, antibody-antigen interaction etc.

The term “protein-based” ligands means herein ligands which comprise a peptide or protein; or a part of a peptide or a part of a protein.

The term “isolation” of an antibody is used herein as embracing purification of a specific product antibody from a mixture comprising other proteins, such as other antibodies, and other components; as well as the separation of an antibody from a product liquid, i.e. to remove an undesired antibody.

DETAILED DESCRIPTION OF THE INVENTION

Thus, the present invention relates to a novel chromatography ligand. The chromatography ligand according to the invention, which is protein-based and of the kind known as affinity ligand, comprises all or parts of Domain C from *Staphylococcus* protein A (SpA). In a first aspect, the present invention relates to a chromatography ligand, which ligand comprises one or more Domain C units from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof. In one embodiment, the present chromatography ligand is substantially alkaline-stable. In this context, the term “substantially alkaline-stable” is understood to mean that the ligand is capable of withstanding repeated cleaning-in-place cycles using alkaline wash liquid without losing its binding capacity.

In a specific embodiment, the present invention is a chromatography ligand, which comprises Domain C from *Staphylococcus* protein A (SpA), but none of the other domains of SpA.

In an alternative aspect, the present invention relates to a chromatography ligand, which ligand comprises one or more Domain C units from *Staphylococcus* protein A (SpA), or a functional fragment or variant thereof, which chromatography ligand is capable of binding to the Fab part of antibodies, as discussed in more detail below.

As discussed above, Jansson et al have already shown that Domain C can act as a separate immunoglobulin adsorbent, not just as part of Protein A. The present inventors have confirmed that the immunoglobulin binding properties of Domain C are fully satisfactory for the use thereof as a chromatography ligand. As also discussed above, Güllich and others had shown that asparagine and glutamine residues in alkaline conditions is the main reason for loss of protein A activity upon treatment in alkaline solutions, and that asparagine is the most sensitive of the two. Consequently, the Domain C ligand, which contains as many as six asparagine residues, was not be expected to present any substantial alkaline-stability as compared to protein A.

However, as shown in the experimental part below, and in FIG. 1, the present inventors have quite surprisingly shown that the SpA Domain C presents a much improved alkaline-stability compared to a commercially available Protein A product (MABSELECT™, GE Healthcare, Uppsala, Sweden) by incubation in alkaline conditions for durations as long as 20 hours. In fact, the Domain C ligand presents values of alkaline-stability which are similar to those of the product marketed as alkaline-stable (MABSELECT SURE™, GE Healthcare, Uppsala, Sweden), wherein asparagine residues have been mutated to other amino acids.

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In addition to this, as discussed above, it has been shown that an especially alkaline-sensitive deamidation rate is highly specific and conformation dependent, and that the shortest deamidation half times have been associated with the sequences—asparagine-glycine- and —asparagine-serine. Quite surprisingly, the Domain C ligand of the invention presents the herein presented advantageous alkaline-stability despite the presence of one asparagine-glycine linkage between residues 28 and 29, using the conventional numbering of the residues of Domain C.

In one embodiment, the ligand according to the invention is able to resist at least 10 hours in 0.5 M NaOH, without deviating more than about 10%, and preferably no more than 5%, from its original immunoglobulin binding capacity. Thus, after 5 hours, it will not deviate more than 10%, preferably 5% from its original binding capacity. In other words, one embodiment of the present invention is a ligand as described above, which after 5 hours incubation in 0.5M NaOH has retained at least 95% of its original binding capacity.

In an advantageous embodiment, the ligand according to the invention is able to resist at least 15 hours in 0.5 M NaOH without losing more than about 20%, and preferably no more than 10%, of its original immunoglobulin binding capacity. In a more advantageous embodiment, the ligand according to the invention is able to resist at least 20 hours in 0.5 M NaOH without losing more than about 30%, and preferably no more than 15%, of its original immunoglobulin binding capacity. In other words, one embodiment of the present invention is a ligand as described above, which after 15 hours incubation in 0.5M NaOH has retained at least 80%, advantageously at least 90% of its original binding capacity.

The skilled person in this field can easily test alkaline-stability by incubating a candidate ligand with sodium hydroxide e.g. as described in the experimental part, and subsequent testing of the binding capacity by routine chromatography experiments.

As easily realised by the skilled person in this field, a chromatography ligand according to the invention may consist of the wild type SpA Domain C amino acid sequence, as shown in SEQ ID NO 1, herein denoted Cwt. In an alternative embodiment, the chromatography ligand according to the invention consists of a functional fragment of SpA Domain C, such as the one shown in SEQ ID NO 2, which discloses a sequence herein denoted Cdel, wherein Asn-Lys-Phe-Asn in positions 3-6 have been deleted as compared to the wild type SpA Domain C sequence. In yet an alternative embodiment, a variant of SpA Domain C is prepared by adding one or more amino acids e.g. to either end of the wild type SpA Domain C amino acid sequence; or by mutation of the wild type SpA Domain C amino acid sequence, provided that such mutation does not substantially interfere with the herein described properties relating to immunoglobulin-binding and alkaline-stability. Thus, in a specific embodiment, the chromatography ligand according to the invention comprises SpA Domain C, as shown in SEQ ID NO 1, which in addition comprises the mutation G29A. Alternatively, the chromatography ligand according to this embodiment comprises the deleted SpA Domain C, as shown in SEQ ID NO 2, which consequently comprises said mutation in position 25 (i.e. G25A). As the skilled person will recognise, such addition, mutation or deletion of amino acids as compared to the wild type sequence should preferably not substantially affect the folding pattern of the SpA Domain C ligand.

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Thus, in one embodiment, the amino acid sequence of the ligand according to the present invention is the sequence defined by SEQ ID NO 1. In a specific embodiment, the ligand according to the invention comprises at least 60%, advantageously at least 80%, more advantageously at least 90% and most advantageously at least 95%, such as about 98% of the amino acids shown in SEQ ID NO 1. In a specific embodiment, the ligand according to the invention comprises at least 35, advantageously at least 46, more advantageously at least 52 and most advantageously at least 55, such as 57, of the amino acids shown in SEQ ID NO 1.

In an alternative embodiment, the amino acid sequence of the ligand according to the present invention is the sequence defined by SEQ ID NO 2. In a specific embodiment, the ligand according to the invention comprises at least 40%, advantageously at least 77%, more advantageously at least % and most advantageously at least 94%, such as about 98% of the amino acids shown in SEQ ID NO 2. In a specific embodiment, the ligand according to the invention comprises at least 31, advantageously at least 42, more advantageously at least 48 and most advantageously at least 51, such as 53, of the amino acids shown in SEQ ID NO 2.

As discussed in the section Background above, methods are readily available for coupling of protein ligands via certain amino acids, preferably amino acids that contain nitrogen and/or sulphur atoms, see e.g. U.S. Pat. Nos. 6,399,750 or 5,084,559. Thus, in one embodiment, the ligand according to the invention further comprises a terminal coupling group, said group preferably comprising one or more nitrogen and/or sulphur atoms. In an advantageous embodiment, the terminal coupling group is comprised of arginine or cysteine. In one embodiment, the coupling group is in the C terminal region.

Further, the present invention also relates to a multimeric chromatography ligand (also denoted a "multimer") comprised of at least two Domain C units, or a functional fragments or variants thereof, as defined above. In one embodiment, this multimer comprises no units originating from SpA. In a specific embodiment, the multimer comprises no other protein-based units. In another embodiment, the multimer comprises no other unit capable of any substantial interaction with a target such as an antibody or a Fab fragment, thus it comprises no other ligand unit. As the skilled person in this field will realise, making a multimer may require adding one or more peptides as linkers between the units. Thus, a multimer limited to containing only Domain C units according to the invention may in addition comprise linkers allowing construction of a multimer wherein each Domain C unit is sufficiently exposed to be able to participate in the binding of target.

In another embodiment, the multimer comprises one or more additional units, which are different from Domain C and preferably protein-based and equally alkaline-stable as Domain C. Thus, in the multimer, the ligand according to the invention may be repeated and/or combined with other units from other sources, such as other proteins. In one embodiment, the multimer is comprised of 2-8 units, such as 4-6 units. In one embodiment, one or more linker sequences are inserted between the multimer units. Such linkers may e.g. be inserted to allow the actual ligand units to maintain their folding pattern. Linkers in this context are well known, and the skilled person can easily decide on suitable amino acids and chain lengths which do not interfere with the herein discussed properties of the ligand. In a specific embodiment, the chromatography ligand according to the invention comprises no other SpA domains than Domain C.

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In a second aspect, the present invention relates to a nucleic acid sequence encoding a chromatography ligand as described above. Thus, the invention encompasses all forms of the present nucleic acid sequence such as the RNA and the DNA encoding the ligand. The invention embraces a vector, such as a plasmid, which in addition to the coding sequence comprises the required signal sequences for expression of the ligand according to the invention. In one embodiment, the vector comprises nucleic acid encoding a multimeric ligand according to the invention, wherein the separate nucleic acids encoding each unit may have homologous or heterologous DNA sequences. This aspect also embraces an expression system comprising a nucleic acid sequence encoding a ligand according to the invention. The expression system may e.g. be a prokaryotic host cell system, e.g. *E. coli* which has been modified to express the present ligand. In an alternative embodiment, the expression system is a eukaryotic host cell system, such as a yeast.

As the skilled person in this field will appreciate, the ligand according to the invention may alternatively be produced by protein synthesis methods, wherein the ligand is obtained by an automated process adding amino acids one at a time following a predetermined sequence. In an advantageous embodiment, segments of amino acids amino acid sequences are synthesized and linked to each other to prepare the ligand according to the invention. Such synthesis and linking procedures are well known to the skilled person in this field.

In a third aspect, the present invention relates to a chromatography matrix comprised of ligands as described above coupled to an insoluble carrier. Such a carrier may be one or more particles, such as beads or irregular shapes; membranes; filters; capillaries; monoliths; and any other format commonly used in chromatography. Thus, in an advantageous embodiment of the matrix, the carrier is comprised of substantially spherical particles, also known as beads. Suitable particle sizes may be in the diameter range of 5-500 μm , such as 10-100 μm , e.g. 20-80 μm . In an alternative embodiment, the carrier is a membrane. To obtain high adsorption capacities, the carrier is preferably porous, and ligands are then coupled to the external surfaces as well as to the pore surfaces. Thus, in an advantageous embodiment of the matrix according to the invention, the carrier is porous.

The carrier may be made from an organic or inorganic material. In one embodiment, the carrier is prepared from a native polymer, such as cross-linked carbohydrate material, e.g. agarose, agar, cellulose, dextran, chitosan, konjac, carrageenan, gellan, alginate etc. The native polymer carriers are easily prepared and optionally cross-linked according to standard methods, such as inverse suspension gelation (S Hjertén: *Biochim Biophys Acta* 79(2), 393-398 (1964)). In an alternative embodiment, the carrier is prepared from a synthetic polymer or copolymer, such as cross-linked synthetic polymers, e.g. styrene or styrene derivatives, divinylbenzene, acrylamides, acrylate esters, methacrylate esters, vinyl esters, vinyl amides etc. Such synthetic polymer carriers are easily prepared and optionally cross-linked according to standard methods, see e.g. "Styrene based polymer supports developed by suspension polymerization" (R Arshady: *Chimica e L'Industria* 70(9), 70-75 (1988)). Native or synthetic polymer carriers are also available from commercial sources, such as GE Healthcare Bio-Sciences AB, Uppsala, Sweden, for example in the form of porous particles. In yet an alternative embodiment, the carrier is prepared from an inorganic polymer, such as silica. Inor-

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ganic porous and non-porous carriers are well known in this field and easily prepared according to standard methods.

In a fourth aspect, the present invention relates to a method of preparing a chromatography matrix, which method comprises providing ligands as described above; and coupling of said ligands to a carrier. In an advantageous embodiment, the coupling is carried out via a nitrogen or sulphur atom of the ligand. In brief, the ligands may be coupled to the carrier directly; or indirectly via a spacer element to provide an appropriate distance between the carrier surface and the ligand. Methods for immobilisation of protein ligands to porous or non-porous surfaces are well known in this field; see e.g. the above-discussed U.S. Pat. No. 6,399,750.

In a fifth aspect, the present invention relates to a process of isolating one or more target compounds, which process comprises contacting a liquid comprising said compound(s) with a chromatography matrix; allowing said compound(s) to adsorb to ligands present on the matrix, wherein said ligands consists of one or more *Staphylococcus* protein A (SpA) Domain C, and/or functional fragments or variants thereof; and, optionally, eluting said compound(s) by the passing across said matrix of a liquid that releases compound(s) from ligands. Thus, in this embodiment, the ligands comprise no other SpA-derived domain than Domain C, or a functional fragment or variant thereof. In an alternative embodiment, said ligands are multimers comprising two or more SpA Domain C units, or functional fragments or variants thereof.

In an advantageous embodiment, the ligands are the ligands described above. The target compound(s) may be any organic compound, biomolecule or other biological material, such as proteins, e.g. antibodies; peptides; cells, such as eukaryotic and prokaryotic cells; nucleic acids, such as DNA, e.g. plasmids, and RNA; virus; etc. In an advantageous embodiment, the target compound(s) is one or more monoclonal or polyclonal antibodies, such as IgA, IgD, IgE, IgG, and IgM. In one embodiment, the target compound is a fragment of an antibody, such as a Fab fragment. In yet another embodiment, the target compound is a fusion protein wherein at least one part is an antibody or an antibody fragment.

In one embodiment, the chromatography matrix is a disposable product, and elution will then not be required if the purpose of the process is to remove the target compound such as the antibody from a product liquid. This embodiment may e.g. be for the removal of an undesired antibody from a liquid, such as a medical liquid or a liquid wherein many antibodies are produced, such as milk from a recombinant animal.

In an alternative embodiment, when the adsorbed compound is the desired product, the elution step is included in the process. To obtain the most suitable conditions for adsorption, a liquid sample is combined with a suitable buffer or other liquid such as water to provide the mobile phase. The present method is advantageously run under conditions conventional for affinity chromatography, and especially for protein A chromatography, as is well known in this field.

In a sixth aspect, the present invention relates to the use of Domain C of SpA, or a functional fragment or variant thereof, as alkaline-stable immunoglobulin adsorbent. In this context, "alkaline-stable" is understood to mean that the adsorbent alkaline-stability is not lower than about 10%, such as about 5%, below that of a commercial products marketed as being alkaline-stable, such as MABSELECT SURE™ (GE Healthcare Bio-Sciences AB, Uppsala, Swe-

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den) during the first 5 hours of incubation in 0.5M NaOH. In an advantageous embodiment, the adsorbent is a ligand as described above. As said MABSELECT SURE™ should present a minimal deterioration after such time and conditions, the antibody binding capacity of the adsorbent should not be lower than about 10%, such as about 5%, below its original binding capacity after such time and conditions. In this context, the term "original" refers to its capacity before any alkaline regeneration, and the comparisons are carried out as side-by-side experiments using a procedure of the herein disclosed kind.

In one embodiment, the use according to the invention comprises a process as described above, wherein the antibodies are eluted from the matrix and which is carried out at least once, such as 2-300 times, optionally with washing steps between; alkaline regeneration of the matrix; and finally repeating said process of isolating antibodies. Washing may e.g. be carried out with a suitable buffer, such as the buffer used to equilibrate the column. In an advantageous embodiment, the regeneration is carried out by incubation with 0.5 M NaOH.

The present invention also embraces a method of purifying one or more target compounds, as discussed above, which method comprises one or more chromatography steps in addition to the purification using the chromatography matrix according to the invention. The method according to this aspect may e.g. comprise a first chromatography step using the present matrix; an intermediate chromatography step using either ion exchange or hydrophobic interaction chromatography (HIC); and finally a polishing step using ion exchange, HIC or reverse phase chromatography. In a specific embodiment, this process comprises a step preceding the chromatography matrix having Domain C ligands as described herein. Such a preceding step may e.g. be a conventional filtration, sedimentation, flocculation or other step to remove cell debris and other undesired components.

In an alternative embodiment, the use according to the invention is an analytical or diagnostic use, such as an immunoassay.

EXAMPLES

The present examples are provided as illustrative purposes only, and should not be construed as limiting the present invention as defined in the appended claims.

Example 1: Column Study of the Alkaline Stability of Four Protein A-Derived Ligands

In this example, the alkaline stability of four chromatography matrices, two of which were comparative and two of which were according to the invention, were tested through a series of chromatographic runs:

MABSELECT™ and MARSELECT SURE™ (both comparative products comprising protein-based ligands, GE Healthcare Bio-Sciences, Uppsala, Sweden), and Cwt (wild type Domain C from SpA, as defined in SEQ ID NO. 1), and Cdel (deleted wild type Domain C from SpA, as defined in SEQ ID NO. 2).

The IgG-binding capacity was measured initially and after incubation steps in 0.5 M NaOH. The incubation times varied from one to five hours, with an accumulated incubation time of 20 hours.

The ligands according to the invention were immobilized on agarose particles according to standard procedure and packed in columns (GE Healthcare). Two of the matrices, MABSELECT™ and MABSELECT SURE™, are commer-

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cial products manufactured by GE Healthcare marketed for the purification of monoclonal antibodies. The ligands of both products are based on the IgG binding *Staphylococcus aureus* Protein A. The MABSELECT™ ligand basically is recombinant Protein A, which consists of five homologous domains (E, D, A, B, C). By comparison, the MABSELECT SURE™ ligand consists of four domains which originate from the domain B analogue “Z”, which in turn has been stabilized against high pH by protein engineering methods. As a result, MABSELECT SURE™ tolerates cleaning-in-place (CIP) conditions of up to 0.5 M NaOH. Both the MABSELECT™ and MABSELECT SURE™ ligands are coupled to agarose particles.

The ligands Cwt and Cdel were constructed as tetramers of identical domains with a C-terminal cysteine residue for coupling to a matrix according to standard procedure.

Materials & Methods

Target Compound

10×10 ml injection liquid, solution, GAMMANORM® 165 mg/ml (Octapharma no. 00 86 64), human normal immunoglobulin, for subcutane infusion or intramuscular injection, was used as the target compound in the chromatography experiments.

Chromatography Columns

Ligand coupling and column packing was carried out as outlined in Table 1 below:

TABLE 1

Columns used in Experiment 1					
Ligand/Matrix	Column ID	Column no.	Batch	Date	Column volume (ml)
MABSELECT SURE™	9	4	U669082	20060310	2.08
Cwt	11	2	U1555055A	20060310	2.02
MABSELECT™	1	7	U1555045A	20060310	2.12
Cdel	13	2	U1555059A	20060303	2.06

“Column ID” refers to a unique number given to each column. These numbers were included in the chromatography methods and can be found in the logbook of the result files. For example, the first column in table 1 was called “MABSELECT SURE™ U669082 Column 4 20060310 (9)”. “Column no.” is the packing number, i.e. columns packed with the same batch of matrix received different Column nos. upon packing. The column volume was estimated by measuring the bed height.

Buffers and Solutions

Buffer A: 50 mM Sodium phosphate, 0.15 M NaCl, pH 7.2

Buffer B: 50 mM Citric acid, 0.15 M NaCl, pH 2.5

Instruments and Laboratory Equipment

Chromatography system: ÄKTA EXPLORER™ 10 (GE Healthcare)

Column hardware: TRICORN™ 5/100 GL (GE Healthcare)

Vacuum degasser: CT 2003-2, 2 channel degasser, ChromTech AB

Spectrophotometer: NANODROP™ ND-1000 Spectrophotometer, NanoDrop Technologies

Centrifuge: Beckman Coulter AVANTI® J-20 XPI with JLA 8.1000 rotor

pH meter (Buffer A): Beckman Φ 360 pH/Temp/mV Meter

pH meter (Buffer B): Laboratory pH Meter CG 842, SCHOTT

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Helium: AGA Gas AB, 10 1 H 20577708, Instrument

Filter for buffer and sample: 75 mm Bottle Top Filter—500 ml, 0.2 µm pore size, Nalgene

Filter for 0.5 M NaOH: 75 mm Bottle Top Filter—500 ml, 0.45 µm pore size, Nalgene

Software

ÄKTA EXPLORER™ 10 was controlled by UNICORN™ 5.01 (GE Healthcare). Apart from controlling the system during the chromatography runs, UNICORN™ was used for method programming and evaluation of the results.

Buffer Preparation

Buffer A: Sodium dihydrogen phosphate and NaCl were dissolved in water. A pH meter was calibrated using pH 4, pH 7 and pH 10 standard buffers. pH was monitored while adding NaOH(aq) to the buffer until pH reached 7.2. The buffer was filtered and degassed with helium prior use.

Buffer B: Citric acid and NaCl were dissolved in water. A pH meter was calibrated using pH 7 and pH 2 standard buffers. pH was monitored while adding NaOH(aq) to the buffer until pH reached 2.5. The buffer was filtered and degassed with helium prior use.

Preparation of 0.5 M NaOH

NaOH(s) was dissolved in water to 0.5 M. The solution was filtered and degassed with helium prior use.

Sample Preparation

Experiment 1

30 ml Gammanorm (165 mg/ml) was diluted to 1 mg/ml with 4950 ml Buffer A. The sample was filtered through 0.2 µm into a sterile 5 litre bottle.

Three 280 nm absorbance measurements were performed on the sample using NANODROP™ spectrophotometer: 1.2573 AU, 1.2432 AU and 1.2101 AU. Mean absorbance: 1.2369 AU.

The absorbance at 280 nm was also measured on ÄKTA EXPLORER™ 10. The sample was pumped with the system pump through the system in bypass mode. A 10 mm UV cell was used and the flow rate was 0.83 ml/min. The absorbance at 280 nm was 1510 mAU. This value was used as a reference when making capacity calculations.

Method Description

Normally, a CIP cycle for MABSELECT SURE™ involves 10-15 minutes contact time of the CIP solution (usually 0.1-0.5 M NaOH). To reduce the amount of CIP cycles in this study, longer contact times were used. The columns were incubated for 1, 2 and 5 hour intervals, with a total contact time of 20 hours. This corresponds to 80 to 120 cycles with 10-15 minutes contact time.

Prior to the CIP incubations two initial capacity measurements were performed per column. After the capacity measurements the columns were incubated in 0.5 M NaOH. After each CIP incubation, one capacity measurement per column was carried out.

Schematically, the experiment was designed as follows:

Two initial capacity measurements per column.

CIP incubation, 1 hour.

One capacity measurement per column.

CIP incubation, 2 hour.

One capacity measurement per column.

CIP incubation, 2 hour.

One capacity measurement per column.

CIP incubation, 5 hour.

One capacity measurement per column.

CIP incubation, 5 hour.

One capacity measurement per column.

CIP incubation, 5 hour.

One capacity measurement per column.

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System Setup:

The experiments were carried out in room temperature. However, the sample was kept on ice to avoid microbial growth. To avoid the formation of air bubbles when the cold sample was heated to room temperature, a degasser was connected between the sample and the pump. The ÄKTA EXPLORER™ 10 was equipped with a 10 mm UV cell for UV detection.

Both buffer and sample was pumped through the system pumps. Following inlets were used:

Sample: B pump (inlet B1)

Buffer A: A pump (inlet A11)

Buffer B: A pump (inlet A12)

0.5 M NaOH: A pump (inlet A13)

Capacity Measurement, Detailed Description

Prior to a capacity measurement (consisting of one capacity measurement per column) sample was pumped in bypass mode, i.e. no column used. The purpose of this was to get “fresh” sample to each capacity measurement and to avoid loading the first volume of sample that remained in tubes and the pump in room temperature during the CIP incubations, onto the first column.

The capacity measurement method for each column consisted of following parts:

Equilibration of the column with 5 column volumes (CV)

Buffer A.

Sample loading. Dynamic binding capacity is determined by loading a sample onto a column packed with the chromatography medium of interest. When the medium becomes more and more saturated with sample, the level of absorbance at 280 nm will increase due to unbound sample passing through the column. In this method, the sample was loaded onto the column until the UV_{280 nm} curve reached 15% of the 280 nm absorbance of the sample.

Wash out unbound sample. The column was washed with Buffer A until the UV_{280 nm} curve dropped below 10% of the 280 nm absorbance of the sample

Elution. Bound material was eluted with 10 CV of Buffer B.

Reequilibration with 5 CV Buffer A.

The flow rate of sample loading was 0.83 ml/min.

CIP Incubation

After each capacity measurement, except for the first of the two initial measurements, a CIP incubation was carried out. In the CIP incubation method, 3 CV of 0.5 M NaOH was pumped through each column at a flow rate of 0.83 ml/min. After this the system was set to pause. The length of the pause depended on the length of the CIP incubation time, i.e. 1 h, 2 h or 5 h. However, the time required for the system to pump NaOH through the columns was subtracted from the pause time. After a CIP incubation 3 CV of Buffer A was pumped through each column at a flow rate of 0.83 ml/min to remove the NaOH. By this procedure, all columns were exposed the same amount of time to NaOH. One more wash cycle with 3 CV Buffer A was finally carried out.

Evaluation of Chromatographic Results

Capacity was determined by measuring the volume of sample applied onto a column until the absorbance at 280 nm reached 10% of the sample absorbance. The dead volumes, i.e. the column volume, mixer and tubing from the pump to the UV cell, were subtracted from this volume. The delay volume without column was determined to 1.02 ml. The capacity values were plotted against the accumulated CIP incubation times. Relative capacity values were achieved by dividing the capacity values after the CIP cycles

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with the mean of the start capacity values. The relative capacity values were used for easier comparisons between the different matrices.

TABLE 2

Results Experiment 1 - Capacity (mg Gammanorm/ml chromatography matrix(gel))				
	MABSELECT SURE™	Cwt	MABSELECT™	Cdel
Start	28.38	27.43	28.98	30.86
Capacity 1				
Start	28.13	27.40	28.98	30.95
Capacity 2				
Capacity after 1 h	29.32	27.79	26.98	30.75
Capacity after 3 h	28.42	27.26	23.08	29.88
Capacity after 5 h	28.25	26.94	20.30	29.25
Capacity after 10 h	27.88	26.07	15.79	26.87
Capacity after 15 h	27.01	24.65	12.52	23.70
Capacity after 20 h	25.93	23.02	10.14	20.35

Experiment 2: Test of Fab-Binding

The Fab-binding ability of the different chromatography media was evaluated in a 96-well filter plate assay. Liquids and chromatography media were mixed on a plate vortex instrument for 1 minute. The bottom of the wells consisted of a filter which retained liquids and the particles of the chromatography media. When subjected to centrifugation, the liquids passed through the filter and were collected in a separate 96-well collection UV-plate attached to the bottom of the filter plate. The absorbance at 280 nm of the collected liquid was measured in a plate reader and used for detection and estimation of Fab. The liquids from different steps, e.g. washing, elution, were collected in different plates and measured separately, to be able to measure the amount of Fab in individual fractions.

10% slurry was prepared of each chromatography medium.

The filter plates were loaded with 200 µl slurry/well, i.e. 20 µl medium/well.

Equilibration—5×200 µl wash in PBS

Sample incubation—100 µl of human polyclonal Fab/Kappa, IgG fragment (Bethyl) in PBS, 15 minutes

Wash—5×100 µl PBS

Elution—3×100 µl 0.1 M glycine, pH 3.0

CIP—2×10 min with 0.5 M NaOH

Analyze plates with liquids UV @ 280 nm

The results of experiment 2 are presented in FIG. 2.

The above examples illustrate specific aspects of the present invention and are not intended to limit the scope thereof in any respect and should not be so construed. Those skilled in the art having the benefit of the teachings of the present invention as set forth above, can effect numerous modifications thereto. These modifications are to be construed as being encompassed within the scope of the present invention as set forth in the appended claims.

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SEQUENCE LISTING

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 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus aureus

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Leu His Leu Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln
 20 25 30

Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
 35 40 45

Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
 50 55

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 1 5 10 15

Asn Leu Thr Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp
 20 25 30

Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala Lys Lys Leu Asn
 35 40 45

Asp Ala Gln Ala Pro Lys
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What is claimed is:

1. A process for isolating one or more target compound(s), the process comprising:
 - (a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:
 - (i) a solid support; and
 - (ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 52 contiguous amino acids of a modified SEQ ID NO. 1, and wherein the modified SEQ ID NO. 1 has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1; and
 - (b) adsorbing the target compound(s) to the ligand;
 - (c) eluting the compound(s) by passing a second liquid through the chromatography matrix that releases the compound(s) from the ligand; and,
 - (d) performing a cleaning in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.
2. The process of claim 1, wherein the amino acid sequence of each polypeptide comprises at least 55 contiguous amino acids of a modified SEQ ID NO. 1.
3. The process of claim 1, wherein the CIP process involves exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.5 M.
4. The process of claim 1, wherein the process for isolating one or more target compound(s) is repeated multiple times such that the total contact time between the CIP solution and the chromatography matrix is at least 5 hours, and wherein the chromatography matrix retains at least 80% of its original binding capacity after the repetitions.
5. The process of claim 3, wherein the process for isolating one or more target compound(s) is repeated multiple times such that the total contact time between the CIP solution and the chromatography matrix is at least 5 hours, and wherein the chromatography matrix retains at least 80% of its original binding capacity after the repetitions.
6. The process of claim 1, wherein the chromatography matrix is capable of retaining at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.
7. The process of claim 1, wherein the chromatography matrix is capable of retaining at least 90% of its original binding capacity after 10 hours incubation in 0.5 M NaOH.
8. The process of claim 1, wherein the chromatography matrix is capable of retaining at least 80% of its original binding capacity after 20 hours incubation in 0.5 M NaOH.
9. The process of claim 1, wherein the target compound is an antibody.
10. The process of claim 1, wherein the ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.
11. The process of claim 1, wherein the ligand binds to the Fab part of an antibody.

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12. The process of claim 1, wherein the ligand comprises a terminal coupling group comprising at least one nitrogen and/or sulfur atom(s).

13. The process of claim 12, wherein the terminal group comprises arginine or cysteine.

14. The process of claim 1, wherein the ligand is coupled to the solid support via thioether bonds.

15. The process of claim 1, wherein the ligand further comprises one or more other alkaline-stable protein-based units.

16. The process of claim 1, wherein the solid support is selected from a polysaccharide, a crosslinked synthetic polymer or an inorganic polymer.

17. The process of claim 1, wherein the solid support is comprised of substantially spherical particles.

18. The process of claim 1, wherein the solid support is porous.

19. The process of claim 1, wherein the solid support is a comprised of substantially spherical particles with a particle size in the range of 20-80 μm and made of a crosslinked synthetic polymer.

20. A process for isolating one or more target compound(s), the process comprising:

(a) contacting a first liquid with a chromatography matrix, the first liquid comprising the target compound(s) and the chromatography matrix comprising:

(i) a solid support; and

(ii) at least one ligand coupled to the solid support, the ligand capable of binding the one or more target compound(s) and comprising at least two polypeptides, wherein the amino acid sequence of each polypeptide comprises at least 55 amino acids in alignment with SEQ ID NO. 1, and wherein each polypeptide has an alanine (A) instead of glycine (G) at a position corresponding to position 29 of SEQ ID NO. 1;

(b) adsorbing the target compound(s) to the ligand; and,

(d) performing a clean in place (CIP) process involving exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.1 M.

21. The process of claim 20, wherein the CIP process involves exposing the chromatography matrix to a CIP solution with a NaOH concentration of at least 0.5 M.

22. The process of claim 20, wherein the process for isolating one or more target compound(s) is repeated multiple times such that the total contact time between the CIP solution and the chromatography matrix is at least 5 hours,

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and wherein the chromatography matrix retains at least 80% of its original binding capacity after the repetitions.

23. The process of claim 21, wherein the process for isolating one or more target compound(s) is repeated multiple times such that the total contact time between the CIP solution and the chromatography matrix is at least 5 hours, and wherein the chromatography matrix retains at least 80% of its original binding capacity after the repetitions.

24. The process of claim 20, wherein the chromatography matrix is capable of retaining at least 95% of its original binding capacity after 5 hours incubation in 0.5 M NaOH.

25. The process of claim 20, wherein the chromatography matrix is capable of retaining at least 90% of its original binding capacity after 10 hours incubation in 0.5 M NaOH.

26. The process of claim 20, wherein the chromatography matrix is capable of retaining at least 80% of its original binding capacity after 20 hours incubation in 0.5 M NaOH.

27. The process of claim 20, wherein the target compound is an antibody.

28. The process of claim 20, wherein the ligand comprises 2-8 of the polypeptides, optionally coupled via linker segments.

29. The process of claim 20, wherein the ligand binds to the Fab part of an antibody.

30. The process of claim 20, wherein the ligand comprises a terminal coupling group comprising at least one nitrogen and/or sulfur atom(s).

31. The process of claim 30, wherein the terminal group comprises arginine or cysteine.

32. The process of claim 20, wherein the ligand is coupled to the solid support via thioether bonds.

33. The process of claim 20, wherein the ligand further comprises one or more other alkaline-stable protein-based units.

34. The process of claim 20, wherein the solid support selected from the group of a polysaccharide, a crosslinked synthetic polymer and an inorganic polymer.

35. The process of claim 20, wherein the solid support is comprised of substantially spherical particles.

36. The process of claim 20, wherein the solid support is porous.

37. The process of claim 20, wherein the solid support is a comprised of substantially spherical particles with a particle size in the range of 20-80 μm and made of a crosslinked synthetic polymer.

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